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STRUCTURE AND DEVELOPMENT OF THE STAMINATE FLOWER AND THE MALE GAMETOPHYTE OF *ENALUS ACOROIDES* (L. fil.), Steud.

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Introduction

In a recent paper on the embryology of *Enalus acoroides* (L. fil.), Steud. (Kausik, 1940 *a*),* mention has been made of the several contributions which have appeared since the publication by Schürhoff (1926) and Schnarf (1931) of the review of earlier literature on the Hydrocharitaceæ. A further addition to this literature has very recently been made, and this deals about the life-history of *Blyxa echinosperma* Hook. f. (Rangasamy, 1941), a previously uninvestigated member of this family.

As long ago as in 1904, Svedelius studied the life-history of *Enalus acoroides*, especially in relation to the phenomenon of pollination; however, his account of some of the important phases in the development of the flower seems to be rather incomplete and many of the details are, therefore, not available. Consequently, the writer began a series of morphological investigations, and the results of some of these have already been published (Kausik, 1940 *a*, *b*). The present paper is a further contribution to the subject and deals about the staminate flowers and the development of the male gametophyte in *Enalus acoroides*.

Material and Methods

Enalus acoroides (L. fil.), Steud. is strictly dioecious. The material for study was collected at Krusadai, a small island in the Gulf of Manaar, South India, where both the male and the female plants grow together on the flat muddy bottom of the sea close to the shore. The material was generally killed in Bouin's fluid, but in a few cases Nawaschin's fluid was also made use of. Entire inflorescences of staminate flowers at various stages of development were dropped into the killing fluids after cutting away the

* By an oversight, Maheshwari's (1933) note on the life-history of *Hydrilla verticillata* Presl. was not referred to in the paper.

outer spathes, especially in the older material, to facilitate proper fixation. After the usual processes of dehydration and infiltration, sections were cut ranging in thickness from 8 to 12 μ, and these were stained either in a 0·5% Heidenhain's iron-alum hæmatoxylin (destaining in 2% iron-alum or in saturated picric acid), or in Newton's iodine-gentian violet (La Cour, 1937).

Structure of the Staminate Flower

The inflorescence bearing the staminate flowers develops completely submerged under water and is held in an upright position by a fairly rigid and well-developed stalk. In each inflorescence there are numerous small staminate flowers, which are all crowded together and are attached to the central axis of the inflorescence by long and slender pedicels (Fig. 1); completely enclosing these flowers during development are two large boat-shaped spathes which later open out slightly as the flowers reach maturity and are about to be detached from the inflorescence. A number of multicellular scales (Fig. 10) are present in the axils of these spathes at the base of the inflorescence (Fig. 1). The scales secrete mucilage during the early stages in the development of the flowers. At this period, the cells of the scales are all filled with abundant cytoplasm and contain prominent nuclei (Fig. 11). In the older inflorescences with almost mature flowers, these cells become depleted of their contents (Fig. 12) and the secretion of mucilage also stops. Goebel (1889–93) recognizes the importance of mucilage secretion and ascribes to it a function of protection for young parts of plants.

Mucilage secreting scales are also found in the pistillate flowers of this plant (Kausik, 1940 *a*, *b*), and they have also been noted very recently in *Blyxa echinosperma* (Rangasamy, 1941). In two other members of this family, *Elodea canadensis* and *Vallisneria spiralis*, similar structures have been figured by Wylie (1904) and Witmer (1937) although no mention of them is made by the authors either in the text or in the descriptions of figures.

The staminate flower of *Enalus* possesses a typical trimerous arrangement of its parts (Fig. 9). The perianth consists of two whorls of floral leaves, an outer of three sepals and an inner of three petals. Both these floral parts pass through identical stages of development and consist also in each case of generally three layers of cells, except at the margins where there are only two (Figs. 13, 14). However, these two sets of floral leaves are somewhat different in structure when fully formed; the sepals show more or less compactly arranged small cells (Fig. 13), while the petals have larger cells, especially towards their inner surface where the epidermal cells become conspicuously papillate (Fig. 16) and contain numerous starch grains. There are also more tannin-cells in the sepals than in the petals. Further, while

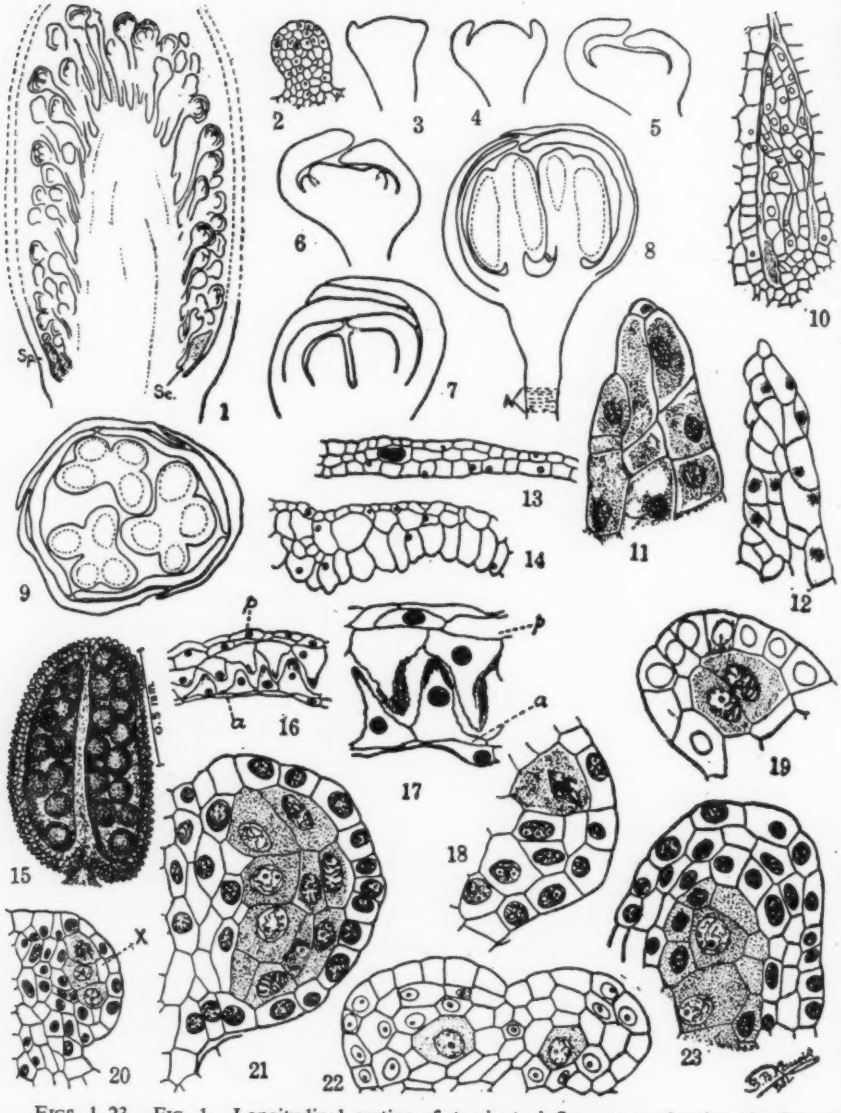
the sepals have imbricating margins, the petals are just valvate (Fig. 9). Thus, notwithstanding any superficial resemblance that is at first evident, structurally these two sets of floral organs are so different that they must be looked upon as distinct morphological entities in the flower.

In *Elodea canadensis* (Wylie, 1904) and *Vallisneria spiralis* (Witmer, 1937; Kausik, 1939) the staminate flowers have only the outer whorl well developed, while the inner is represented by rudimentary structures. Witmer (1937) has shown that the rudimentary petal is single in the American form of *Vallisneria* and that it is placed on the side of the flower opposite that where a rudimentary stamen is present. A similar situation is also seen in the Indian plant (Kausik, 1939), although this feature has not been mentioned in an earlier paper on its life-history by Rangasamy (1934).

There are three stamens, each with a massive anther and an extremely short filament (Fig. 15). The stamens are found in an upright position in the centre of the flower where they form a compact group (Fig. 8). It is interesting to note that a parallel case with similarly upraised stamens is seen in the American *Vallisneria* (Wylie, 1917), while in both the European (Kerner, 1891; Svedelius, 1932) and the Indian forms (Rangasamy, 1934; Kausik, 1939), the stamens are widely divergent and directed obliquely outwards. The significance of these structural differences is pointed out in the concluding part of this paper.

When the staminate flowers reach maturity, they are detached from the axis of the inflorescence and rise to the surface of water owing to the buoyancy caused by a bubble of air enclosed between the floral parts. On the surface of water the sepals and petals snap back and become recurved, so that the three stamens are completely exposed. Wylie (1917) states that in the long-stalked staminate flowers of *Elodea iænsis* " a bubble of gas is generally associated with the partly opened sepals, giving extra buoyancy to the submerged flowers, which tug at their anchorage like captive balloons". According to the same author the released staminate flowers of *Elodea canadensis* (1904) " dart to the surface and there freely explode, scattering their pollen on the surface of water " (1917), while in *Vallisneria, spiralis* (1917) the staminate flowers rise slowly to the surface of water where they very slowly open.

Svedelius (1904) reports that the staminate flowers of *Enalus acoroides* reach the surface of water exclusively during low tide. He remarks: " The male flowers are like balloons when closed. When ripe they are filled with gases, and the sudden recurving of the sepals and petals may apparently be due to the pressure of the gas bubbles on reaching the air. As the



FIGS. 1–23.—FIG. 1. Longitudinal section of staminate inflorescence showing the flowers attached to the central axis and the enclosing spathes ; the dotted lines denote that the spathes have been cut away ; *Sp.*, spathe, *Sc.*, mucilage secreting scale. FIGS. 2–7. Stages in the development of the floral parts. FIG. 8. Outline of longitudinal section of old flower bud ; the cross zone in the upper part of the pedicel is marked A. FIG. 9. Outline of transverse section of flower bud. FIG. 10. Mucilage secreting scale. FIG. 11. A portion of scale enlarged. FIG. 12. Scale at a much later stage. FIGS. 13 and 14. Portions of transverse sections of sepal and petal

respectively. FIG. 15. An entire anther mounted whole to show the papillate epidermis and the large pollen grains. FIG. 16. The papillate epidermal cells of the anther and petal to show dovetailing arrangement. FIG. 17. Papillate epidermal cells of anther and petal enlarged to show the folds in the wall ; in this and also in FIG. 16, *p*. petal, *a*, anther. FIG. 18. Part of longitudinal section of anther showing one locule containing a single large archesporial cell which is in division. FIG. 19. Later stage showing two cells. FIG. 20. Longitudinal section of one locule showing the row of sporogenous cells ; a parietal cell is formed at X. FIG. 21. Later stage showing the parietal layer and the separation of the tapetum. FIG. 22. Transverse section of anther at about the same stage as in FIG. 21 ; only two locules are shown. FIG. 23. Much later stage showing the endothecium, the single middle layer, the tapetum, and the spore mother cells. FIG. 1. × 12. FIGS. 2–7. × 80. FIGS. 8–9. × 40. FIG. 10. × 120. FIGS. 11 and 22. × 400. FIGS. 12–14 and 16. × 200. FIG. 15. × 35. FIGS. 17–19, 21 and 23. × 450, and FIG. 20. × 270.

perianth, within certain limits, can easily be more or less pressed together, the explanation of this periodical phenomenon may be the following. With increased pressure, that is during high water, the buds are somewhat compressed, and consequently the volume diminishes, but since their absolute weight is the same it follows that the specific gravity is increased and consequently the power of attraction upwards diminished. With diminished pressure again, during low water, the volume of the buds is augmented and thus the specific gravity diminished, so that the power of attraction upwards is augmented. The chance for the male flowers to rise to the surface during low water is consequently greater than during high water, in otherwise similar circumstances."

Origin and Development of Floral Parts

The primordium of the staminate flower arises on the axis of the inflorescence as a spherical undifferentiated mass of cells (Fig. 2). Gradually two distinct regions become clearly marked out in this primordium, an enlarged terminal portion and a narrow lower portion (Fig. 3). The former then forms the primordia of the different floral organs, while the latter gives rise to the pedicel of the flower. The primordia of the three sepals are the first to arise and these appear along the periphery of the terminal part of the flower primordium (Fig. 3). The sepals grow and very soon bend over a central hemispherical mound of tissue (Fig. 4) which later becomes more or less flattened (Fig. 5). In this flattened region the primordia of the petals become next evident (Fig. 6), and lastly the stamens are differentiated from the tissue which still remains in the centre of the young flower bud (Figs. 6, 7). The stamen primordia grow for a time more rapidly than those of the petals, but the latter very soon overtake the former in development and thus bend over and enclose the stamens. By this time, the sepals are fully formed and cover the inner floral organs (Figs. 7, 8).

When the floral parts are passing through the stages of development, the narrow lower part of the flower which forms the pedicel increases rapidly in length and becomes slender. Later, when the flower is almost mature and is about to be released from the inflorescence, a definite cross zone of regularly arranged cells (Figs. 8 at A, 27) is seen in the upper part of the pedicel immediately below the base of the flower. This region is devoid of tannin-containing cells which are particularly conspicuous elsewhere in the pedicel and also in the floral parts. The detachment of the flower is effected later at this cross zone.

Development of the Anther

During the early development of the stamen primordium, there is first seen only a more or less uniform increase in size, but after a time a clear distinction becomes apparent between an upper part which forms the massive anther and a lower part which develops into the short filament. With further development of the upper part, a single large hypodermal cell, the archesporium, which is large in size and contains rich cytoplasm and a prominent nucleus, can be seen at each of the four corners corresponding in position to an anther locule in transverse sections. Even in longitudinal sections of the anther at this stage there is but a single such cell (Fig. 18), and it is thus evident that the archesporium in each locule consists of only one cell. This cell next undergoes a few divisions to form a short row of some three to five cells disposed parallel to the long axis of the anther (Fig. 20). Later each cell of this row divides once periclinally, after which an outer layer of smaller cells becomes separated from an inner of larger cells with rich contents (Figs. 20 at X, 21). The former is the primary parietal layer and the latter the sporogenous layer. The primary parietal layer next forms two layers by a periclinal division (Fig. 21), and of these the inner becomes the tapetum, while the cells of the outer layer undergo one more periclinal division to give rise to two layers outside the tapetum (Fig. 23). The outer of these layers is the endothecium and the inner forms the single middle layer in the wall of the anther.

While these changes are taking place in the wall of the anther, the sporogenous cells, forming as already noted a row of three to five cells, increase in size, become more and more prominent, and ultimately form the microspore-mother cells (Fig. 23). Their nuclei enter the meiotic prophasic stages, some of which are represented in Figs. 28 to 31. Each of these prophasic nuclei contains one or more nucleoli (Figs. 28, 29), as many as four in some cases (Fig. 30). The nucleoli include a number of small vacuoles, a feature especially clearly seen in gentian violet preparations.

At the end of the prophasic stages the first division spindle is formed in the mother cells, and on completion of division two daughter nuclei are organized, separated by a delicate equatorial wall (Fig. 32) to form two cells. The second division spindles are next formed in the daughter cells and these are disposed parallel to each other (Fig. 33). Thus the formation of the microspore tetrads is successive and the arrangement isobilateral as in many other members of this family. In *Ottelia alismoides*, however, tetrahedral and linear arrangements are also seen, in addition to the isobilateral condition (Narasimha Murthy, 1935).

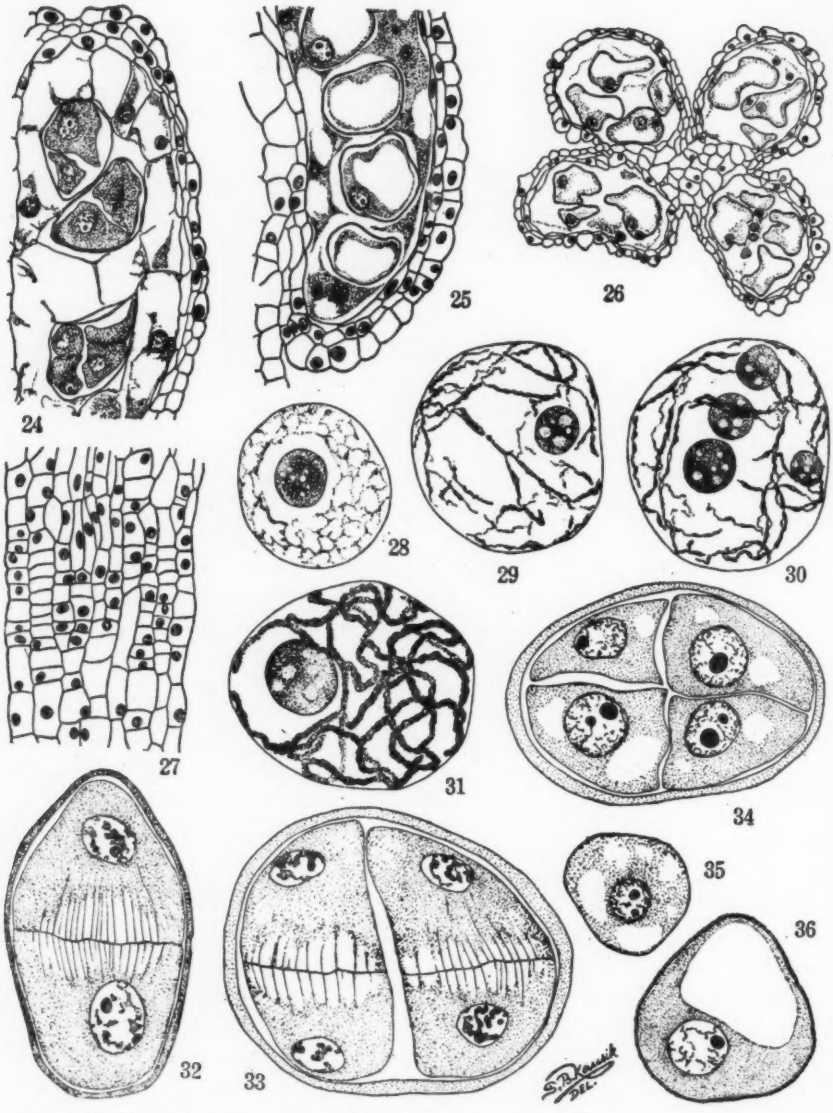
For a time the microspores remain within the mother cell wall, which in the meanwhile swells up on account of gelatinization (Figs. 32–34). Gradually, the wall undergoes complete dissolution and the microspores are thus set free to form the pollen grains (Fig. 25) which become spherical in shape and increase in size (Figs. 35, 36).

At the time of tetrad formation certain changes are seen in the anther wall. The inner and lateral walls of the tapetal cells disappear (Fig. 24), and the cell contents are released into the locule (Fig. 25) to form a common mass of cytoplasm with free nuclei. This is the tapetal periplasmodium, which is also seen in the other previously investigated members of this family,[4] except in the Indian form of *Vallisneria* (Rangasamy, 1934), where a well organized tapetum is said to be absent and no mention is, therefore, made about the formation of the plasmodium.

The tapetal plasmodium becomes intrusive, the cytoplasm alone first moving into the spaces between the pollen grains, but later followed by the migration of the nuclei also (Figs. 25, 26). An intimate contact is thus established between the developing pollen grains and the plasmodium which is used up gradually, partly as nutriment and partly for building up the the spine-like outgrowths seen on the exine of the pollen grains (Figs. 36–44).

The formation of the tapetal periplasmodium is well known in the Commelinaceæ in the works of Tischler (1915), Mascré (1925), and others; here the tapetal nuclei exhibit changes in form and structure (Tischler, 1915), and also increase in number by regular mitoses (Lakshminarasimha Murthy, 1938). Neither of these phenomena occurs in the case of *Enalus acoroides*.

When the anthers are mature, the epidermal cells of the wall become papillate (Figs. 15-17) as in the case of the petals. The papillate cells of both the anthers and petals become dove-tailed with each other (Figs. 16, 17) on account of mutual contact and pressure during development. Further, the papillate epidermal cells also form numerous minute parallel folds in their walls. Similar, but much less conspicuous folds are also seen in the walls



FIGS. 24–36.—FIG. 24. Part of longitudinal section of anther showing tetrads of microspores (one tetrad is not seen here), the tapetum with the walls breaking down, the middle layer, the endothecium, and the outer epidermis. FIG. 25. Later stage showing rounded pollen grains, tapetal periplasmodium, the crushed cells of the middle layer, the endothecium, and the epidermal cells becoming enlarged. FIG. 26. Transverse section of an older anther; the epidermal cells are

papillate and become more so later, and the middle layer is almost completely lost. FIG. 27. Upper part of pedicel to show the regular arrangement of small cells at the cross zone, enlarged from Fig. 8 where this regon is marked X. FIGS. 28–31. Prophasic stages of the nucleus of the microspore-mother cell. FIG. 32. Late telophase of first division showing equatorial plate to form daughter cells. FIG. 33. Late telophase of second division showing parallel arrangement of spindles and the formation of the tetrad. FIG. 34. Tetrad of microspores within the mother cell wall; the wall of the mother cell is thick on account of gelatinization in this and the two previous figures. FIG. 35. Young pollen grain. FIG. 36. Later stage with a large central vacuole and the nucleus pushed towards one side ; the exine shows the minute spines. FIGS. 24 and 25. × 270. FIG. 26. × 120. FIG. 27. × 200. FIGS. 28-30. × 2700. FIG. 31. × 1800. FIGS. 32–34. × 600. FIG. 35. × 400, and FIG. 36. × 450.

of the epidermal cells on the apposed inner and outer surfaces respectively of the sepals and petals. Svedelius (1904) has noted the presence of these folds only in the walls of the epidermal cells of the anthers. In this connection it may be mentioned that the epidermal cells of the anther possess peculiar thickenings in *Vallisneria spiralis* (the Indian form), and it is stated that the anther wall becomes rigid on account of these thickenings and that the pollen grains are shed by the bursting of the wall (Rangasamy, 1934).

The endothecium does not develop well, and the so-called fibrous layer is, therefore, not formed. The middle layer of the wall becomes very much crushed during the enlargement of the pollen grains, and is finally completely obliterated in the mature anther (Figs. 16, 25, 26).

The Male Gametophyte

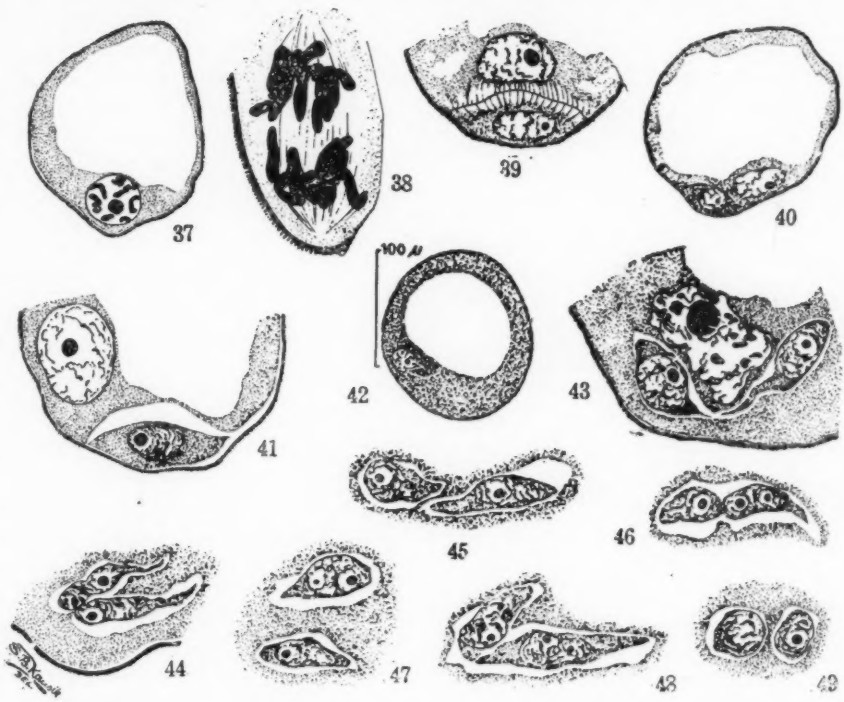
The developing pollen grains are very large and measure as much as about 150 μ in diameter (Fig. 42). There are only a very few of these in each anther locule (Fig. 15). Svedelius (1904) reports that the output of grains per locule is about 30. But probably the number is much less, for, as already noted, there are only about three to five mother cells in a locule, and if all of them developed, the number is not over 20. The total output per anther was found to be 56 in an actual count of the pollen grains removed from an entire teased out preparation of the anther.

In the wall of the pollen grain the intine is not developed, but the exine is well formed and is closely beset on the surface with numerous minute spines (Figs. 36–44). Svedelius (1904) states that the exine is smooth and that *Enalus* in this respect resembles many other water plants. Wylie (1904) has noted the spines in *Elodea canadensis* where they " play an important part in the process of pollination ". Similar spine-like outgrowths are found in *Vallisneria spiralis* (Rangasamy, 1934; Witmer, 1937) and *Blyxa echinosperma* (Rangasamy, 1941). In *Ottelia alismoides*, however, the pollen grain is figured with a smooth, but well-developed exine devoid of spines (Narasimha Murthy, 1935).

The nucleus of the pollen grain is at first situated in the centre with a number of vacuoles all round (Fig. 35). Later the nucleus is pushed towards one side on account of the formation of a large central vacuole (Fig. 36), which subsequently enlarges very much so that the cytoplasm of the pollen grain is formed into a thin layer all round except at one side where the nucleus becomes located (Fig. 37). The nucleus now undergoes a mitotic division to form two daughter nuclei (Figs. 38, 39), of which the one formed towards the inside of the grain is much larger than the other near the wall (Fig. 39). These are respectively the tube and the generative nuclei. The telophase spindle for this division is short and wide, and shows the formation of a delicate curved wall at the equatorial region, so that finally the generative nucleus is cut off as a small lenticular cell, the generative cell, and the rest of the pollen grain retains the tube nucleus (Figs. 39, 40). The delicate wall later disappears leaving a narrow clear area in its place (Fig. 41). Witmer (1937) has noted a similar condition in *Vallisneria spiralis*. Wulff and Maheshwari (1938) remark: "The question whether the formation of a cell-plate in this division is also followed by a real cell wall still remains an open one. Usually the generative cell is seen to be separated from the vegetative by a clear concave space and if a cell wall is formed at all it must be of a very transitory nature".

In some plants a definite position is seen in the pollen grain in which the division just referred to takes place, and it is also claimed that this is constant for a species, or sometimes a whole genus (*cf.* Wulff and Maheshwari, 1938). Wylie (1904) has observed the division on the outer side of the pollen grain in *Elodea canadensis*. But in *Enalus acoroides* the pollen grains become free from one another and assume a spherical shape long before the division occurs so that no indication is seen of the position where the spindle is formed. As Wulff and Maheshwari (1938) remark, there must "remain a host of cases in which the pollen grains round up at a very early stage and it becomes impossible to distinguish the outer pole of the pollen grain from the inner."

During the further development of the male gametophyte in *Enalus acoroides*, the tube nucleus increases very much in size and assumes a very irregular outline forming a number of peripheral lobes all over, as almost to suggest that there may result a fragmentation (Fig. 43). Actually, however, this does not occur and the tube nucleus remains intact till the end. In the meanwhile, the generative cell gives rise to two small spindle-shaped male cells situated in the interior of the pollen grain (Figs. 42–49). The actual division resulting in the male cells could not be seen in the material examined.



FIGS. 37–49.—FIG. 37. Pollen grain nucleus about to divide. FIG. 38. Anaphase of division; the spines on the exine are clearly shown here. FIG. 39. Late telophase showing the delicate curved wall to form the generative cell. FIG. 40. Pollen grain with the lenticular generative cell and the tube nucleus lying in the body of the grain. FIG. 41. Later stage showing the narrow clear space separating the generative cell. FIG. 42. Entire pollen grain mounted whole and showing the tube nucleus, the spindle-shaped male cells, and the starch grains scattered in the cytoplasm. FIG. 43. Part of pollen grain showing the tube nucleus with a very irregular outline, and the two male cells. FIGS. 44–48. Male cells in various positions ; a clear area is present all round the male cells. FIG. 49. Male cells represented in transverse section. FIGS. 37 and 40. × 450. FIG. 38. × 1800. FIGS. 39, 41 and 43–49. × 900, and FIG. 42. × 350.

In the male cells the nucleus is usually much longer than broad, and most frequently dumb-bell-shaped (Figs. 44, 45, 48). Sometimes one end of the nucleus appears to be rather narrow, while the other end is broader and also rounded (Figs. 45, 46, 48). Investing the nucleus the male cell has always a thin layer of cytoplasm, which is especially clearly seen at the ends of the cell where it forms a tail-like prolongation (Figs. 43–48), and in several cases only one end is thus produced (Figs. 44, 46). All round the male cells there is invariably seen a definite clear space, by which the nature of the cells can be easily recognized.

The two male cells in a grain appear in many cases to be connected together by their ends (Figs. 44, 46, 48), but the fact that they frequently also lie far enough apart (Fig. 47) precludes the possibility that they must always remain united in this manner. In fact, the two male cells assume the most varied positions and thus present many different arrangements as shown in Figs. 43 to 48. In Fig. 49 depicting the two cells in a transverse section, the thin layer of cytoplasm investing the nuclei at the sides is shown.

Thus the pollen grains of *Enalus acoroides* become three-celled at the time of shedding. They also contain large quantities of starch grains which are scattered throughout the cytoplasm, and Svedelius (1904) notes that these make the grains heavier so that they sink in sea water.

Conclusions

Wylie (1904), in his work on *Elodea canadensis*, remarks that the Helobiales include an ascending series of forms which find their climax in the Hydrocharitaceæ, and further observes in a subsequent paper on *Vallisneria spiralis* (1917) that the devices here favouring pollination through the agency of the surface film bespeak long association with water and aquatic conditions. The writer (1939) has elsewhere discussed the importance of this surface film in the pollination of the Indian form of *Vallisneria*. Svedelius (1904) notes in *Enalus acoroides* that when an associated group of pistillate and staminate flowers is submerged under water, the petals of the former " at once fold together owing to the pressure of the water ", and the staminate flowers are thus " enclosed by the embracing petals which shut rather closely together. Thus it....occurs that the anthers get an occasion to drop their pollen and since this is heavier than the water it sinks down and can scarcely escape falling upon the stigmas, which stand *vertically in a circle*, owing to the *changed position underneath the level of the water*." He further observes that no transfer of pollen from the staminate flowers caught in the folds of the petals of the pistillate flowers can take place directly on the surface of water, " because the stamens, owing to their lack of filaments and to their straight position ", can scarcely reach the stigmas " even if they were brought far in towards the centre " of the pistillate flowers. In the pollination of the American form of *Vallisneria*, Wylie (1917) draws attention to the fact that, in addition to the agency of the surface film of water, any degree of submergence of the groups of pistillate and staminate flowers is helpful, but that " complete submergence .... is not necessary to adequate pollination "; the two stamens are here united together at the base and are also, as in *Enalus*, quite erect in the centre of the flower. This is sharply contrasted with the situation noted for the European (Kerner, 1891 ; Svedelius,

1932) and the Indian forms (Rangasamy, 1934; Kausik, 1939) of *Vallisneria* where the two stamens are divergent and protrude beyond the margins of the flower. These different features seen in the staminate flowers of *Vallisneria* and *Enalus*, and also the differences in the structure and arrangement of parts in their corresponding pistillate flowers, probably account for the different modes of pollination noted for these plants.

The papillate epidermal cells of the recurved petals and anthers, and the minute folds seen in their walls, probably help to prevent wetting of these floral parts. In addition, they may also be of advantage in lodging the staminate flowers securely in the folds of the petals of the pistillate flowers. Svedelius (1904) observes: "When the male flowers arrive floating on the surface of the water they very easily get caught upon the petals, especially the folded edges", of the pistillate flowers when "one may observe little rows of captured male flowers along the edges, which hold them fast in spite of wind and currents."

In conclusion, it must be remarked that, notwithstanding the structural simplicity of the staminate flowers, *Enalus acoroides* exhibits a high degree of specialization, especially in relation to the phenomenon of pollination. The first step in this is effected when the pistillate and staminate flowers become associated together in groups at the upper limit of water through the influence of the surface film, and the subsequent events take place when these groups are submerged under water. A comparison of the events in pollination in *Enalus acoroides* with those in *Elodea canadensis* and *Vallisneria spiralis* provides a remarkable series of structural adaptations to the surface conditions. At one end of this series are forms like *Elodea canadensis* and the Indian form of *Vallisneria* where the actual transfer of pollen takes place directly on the surface, while at the other end is *Enalus acoroides* in which the pollen is conveyed to the stigmas only under water. The American *Vallisneria* seems to occupy an intermediate position in this series, for, as Wylie (1917) has shown, any degree of submergence is helpful, although the cup-like depressions of the surface film alone may be sufficient to bring about contact between the stigmatic lobes of the pistillate flowers and the dehisced anthers bearing clusters of large pollen grains in the staminate flowers.

Summary

In the male plants of *Enalus acoroides* (L. fil.) Steud., the inflorescence develops under water and bears innumerable tiny staminate flowers attached by means of long and slender pedicels. Two large boat-shaped spathes are found covering these flowers during development, and these open out

partially later when the flowers reach maturity and are about to be released from the inflorescence.

Prominent mucilage secreting scales are found in the axils of the spathes.

The staminate flowers are typically trimerous and consist of three sepals, three petals, and lastly three erect stamens, and the primordia for these floral parts arise in the same order.

The epidermal cells of the anthers and those on the inner surfaces of the petals become conspicuously papillate and are also dove-tailed with each other. Their walls form numerous minute folds. Similar, but less prominent, folds are also seen in the walls of the epidermal cells on the inner and outer surfaces of the sepals and petals respectively.

A definite cross zone of regularly arranged cells is seen in the upper part of the long pedicel; later, the detachment of the flower takes place at this region.

At first there is a single large hypodermal archesporial cell for each locule of the anther. Later this cell forms a row of some three to five cells which divide periclinally and form an outer primary parietal layer and an inner sporogenous layer. The former undergoes further divisions to form the tapetum, a single middle layer, and the endothecium. The so-called fibrous layer is not formed.

The tapetal cells break down at the time of tetrad formation to give rise to the tapetal periplasmodium which becomes intrusive.

The tetrads of microspores are formed according to the successive method and are arranged in an isobilateral manner.

The developing pollen grains become very large in size, about 150 μ in diameter, and are spherical in shape. The intine is not formed, but the exine is well developed and is beset on the surface with minute spines.

The pollen grain at the time of shedding consists of three cells, two small spindle-shaped male cells disposed in various manners inside the cytoplasm of the grain, and the tube cell which fills the grain completely and shows a large tube nucleus with an irregular outline. In addition, there are numerous starch grains scattered throughout the cytoplasm of the grain.

Some important structural features of the staminate flowers are discussed in the paper; the process of pollination in *Enalus acoroides* is compared with that in some other members of this family.

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STUDIES IN *PARA-SORGHUM* SNOWDEN—THE GROUP WITH BEARDED NODES*

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Stapf (1917) separated the group Sorghum from the heterogeneous *Andropogon* of the earlier workers and gave it an independent generic stand.[1] Snowden (1936) further classified this genus into two sub-sections—(i) *Eu-sorghum*, (ii) *Para-sorghum*.[2]

The *Para-sorghums* are distinguished from the *Eu-sorghums* (true sorghums) by the bearded nodes and the reduced number of chromosomes being $2n = 10$ as against $2n = 20$ in the latter. The sub-section *Para-sorghum* consists of four known species.

(i) *Sorghum purpureo-sericeum*, Ascher et Schweinf.
(ii) *S. dimidiatum*, Stapf.
(iii) *S. versicolor*, J. N. Anderss
(iv) *S. nitidum*, Pers.

These species are the least known among the sorghums and they have been studied little so far. This paper deals with the affinities, inter-relationships and the differences between the species in the group.

Origin and Distribution

The *Para-sorghums* extend in their distribution from Africa eastwards to India, China and the Malayan peninsula and southwards to Australia.[3] In Africa itself they are found to occur in well defined geographic zones. Thus *S. purpureo-sericeum* is found on the eastern side of the Blue Nile and *S. dimidiatum* on its western side, while *S. versicolor* is distributed more towards the south-eastern portion of Africa.[4] Outside Africa *S. purpureo-sericeum* has been reported only from Central India. *S. nitidum* is distributed

* This is the third of the series of articles on groups of Sorghum. The first on *S. sudanense* and the second on *S. halepense* were published in the *Proc. Ind. Acad. Sci.*, Oct. 1939 and March 1941 respectively.

in the southern portion of Asia and tropical Australia, and has not so far been found in Africa.[5]

The materials for the present study were secured from different sources. *S. purpureo-sericeum* and *S. dimidiatum* were obtained through Kew, *S. versicolor* from Texas, U.S.A., and *S. nitidum* was collected from the western ghats in the Coimbatore district (South India).

Agro-Botanical Description[6]

Habit.—Annuals under south Indian conditions. *S. nitidum* is the only species with a perennial tendency ; *Duration*—120 to 150 days ; *Tillers* 15 to 30 ; *Bloom* almost nil ; *Seedling*—coleoptile purple ; leaves—bluish green ; tillers in seedling stage, spreading ; *Mature plant—Nodal band* purple, bearded, hairs white; *Leaf-sheath* does not take up pigment ; axil of leaf sheath above the nodal band, purple ; *Leaf blade*—narrow ; margins not wavy; *Midrib*—white ; *Leaf junction*—purple, bearded, hairs white ; *Panicle*—well emerged, 10 to 15 cm. above the subtending leaf ; branches bending to one side, bearded at the nodes, pulvinate ; *Peduncle*—surface undulating 30 to 40 cm. long; *Spikelets*—cymosely arranged, both pedicelled and sessile spikelets deciduous, pedicelled spikelets no purple wash ; *Glumes* of sessile spikelets very thick, coriaceous, reddish or blackish purple, unbleached ; *Awn* very long ranging from 30 to 40 mm. In *S. nitidum awn* may or may not be present and when present only 9 mm. long. *Lodicules* highly ciliate ; *Anther* yellow with purple wash when fresh, and turn reddish-brown on drying. *Stigma* yellow with a brown wash ; *Grain* brown, pearly and enclosed completely and tightly within the glumes.

Chief Characteristics

The group as a whole is characterised by a dense hairiness. The coleoptile is glabrous but the hairiness is manifested from the first foliar leaf. Three kinds of hairs can be distinguished. The first kind are the soft short hairs. These are about 0·5 mm. in length and densely cover the leaf-sheath and the blades giving the plants a velvety touch and an ashy green look. Long hairs (about 5–7 mm.) with tubercles at the bases, form the second type, these hairs being distributed sparsely over the leaf-sheath and blade. In *S. purpureo-sericeum* and *S. dimidiatum* these long hairs are confined mostly to near about the leaf junction on the upper surface of the leaf blade and the upper half of the leaf sheath. In *S. versicolor* and *S. nitidum* the hairs are distributed evenly all over the leaf sheath and blade. The third type consists of hairs which are also long but without tubercles at the bases. These form beards at the nodes, leaf junctions, bases of panicle branches and at the base of the

spikelets. On the node these long hairs radiate uniformly all round presenting a disc-like appearance (Fig. 3). The hairs are white and are fairly uniform in length (6-7 mm.). At the base of the node, these hairs are very short and pubescent. The hairs at the leaf junctions and at the base of the panicle branches have no definite arrangement but are merely clustered in bunches. The hairs at the basal portion of the spikelets are about 3 mm. long. These are arranged all round the basal portion but unlike the hairs on the nodal band these are adpressed to the glume and in addition pigmented.

Unlike the *Eu-sorghums* these do not have well developed adventitious roots nor do they put forth side shoots from the top nodes (Fig. 1). Axillary buds are present but remain dormant. Even the lower leaf-sheaths and blades remain green till complete ripening and hence do not take up pigment so prominently as in the *Eu-sorghums*.[7] The dried leaves however take on a reddish tint, similar to the sap colour of the cultivated sorghums.[8]

Tillering commences even when the seedlings are about a fortnight old. As though in compensation to the suppression of the side shoots, there is a free increase in the production of tillers.

The primary branches of the panicles are not divided to the same extent as in the *Eu-sorghums*. It has already been reported that in the *Para-sorghums* the glumes emerge green in colour from the boot but turn purple the next day.[9] Anthesis occurs between 8–30 and 9–30 A.M.[10] Both the pedicelled and sessile spikelets are shed at the dough stage of the grain itself, *i.e.*, 3 to 4 weeks after flowering. Unlike *S. halepense* (where about 10 per cent. of the sessile spikelets persist)[11] all the spikelets are shed leaving the panicle branches bare. The glumes are thicker and more coriaceous than in the *Eu-sorghums* and do not get bleached even if there is no setting of grain. The awns are very long in this group and the longer awns have been associated with the larger spikeletted species.[12] The seeds require a resting period of one year for good germination. Even after this resting period the seeds germinate only with a second spell of rain or a second irrigation, as the spikelets take a long time to get fully soaked. The grains although brown exhibit their pearly nature clearly.[13] Unlike the cultivated sorghums the topmost layer can be easily peeled off showing the corneous endosperm. The shape of the grain conforms to the shape of the glumes. This is seen very distinctly in the case of *S. dimidiatum* where the top half gets tapered rather abruptly.

The differentiating characters of the four *Para-sorghums* are given in the following table.

Character	*S. purpureo-sericeum*	*S. dimidiatum*	*S. versicolor*	*S. nitidum*
Glume size ..	8·0 ×3·0 mm.	7·0 ×2·5 mm.	6·5 ×2·5 mm.	3·0 ×1·5 mm.
„ texture ..	Fully coriaceous	Coriaceous at the base and papery at the top	Fully coriaceous	Fully coriaceous
„ tip ..	Broadly mucronate	Acute	Acute	Acute
„ colour ..	Reddish purple	Reddish purple	Blackish purple	Blackish purple
„ hairiness ..	Top edges hairy, base bearded	Top edges hairy, base bearded	Completely covered with hairs, base bearded	Completely covered with hairs, base bearded
„ hair length	2·0 mm.	2·0 mm.	2·0 mm.	0·5 mm.
„ hair colour	Brownish purple	Brownish purple	Brown	Brown
Awn length ..	35–40 mm.	32–37 mm.	30–35 mm.	Present or absent. When present 7–9 mm.
Plant height ..	100–120 cm.	100–120 cm.	100–120 cm.	40–50 cm.

From the above table it will be seen that *S. purpureo-sericeum* is more allied to *S. dimidiatum* and *S. versicolor* to *S. nitidum*. In Fig. 4 are presented spikelets of these four species.

S. purpureo-sericeum.—The word *purpureo-sericeum* refers to the colour and the texture of the beard at the base of the spikelets—purple and silky—at the flowering stage. This species can be distinguished from *S. versicolor* and *S. nitidum* by the larger spikelet which is non-hairy as well as reddish purple in colour. The shape of the glume tip is very peculiar in being broadly mucronate. There are in this group two distinct types of pigmentation in the pedicelled spikelets. In the first type which forms the majority, the pedicelled spikelets have no purple wash at the flowering time, and this non-pigmented condition persists until maturity. In the second, which has occurred as a variant in the first group, both the involucral glumes of the pedicelled spikelets have a purple wash close on emergence from the boot. This pigment is well marked during the flowering stage and fades as the spikelets mature. In a cross between these two types the F_1 plant had a purple wash which in the F_2 generation gave 49 plants with purple wash and 15 plants with no purple wash. The F_3 conformed to the F_2 behaviour giving a total of 44 plants, with purple wash and 15 plants with no purple wash indicating a simple dominance of the purple wash. Types with purple

wash on the pedicelled spikelets have been met with in *S. sudanense* and *S. dochna* also where it behaves as a simple dominant.[6] But the purple pigment was seen only on one of the glumes which was exposed to the sun. Janaki Ammal has recently recorded that the 2*n* chromosome number in this species ranges from 10 to 14.[14]

S. dimidiatum.—The word dimidiatum means " halved " and this species takes its name from the lower glume being coriaceous upto half its length and then getting abruptly papery. Hence the lower portion takes up a reddish purple pigment and the papery upper portion is incapable of taking up pigment. The upper glume is as long as the lower, coriaceous throughout and the whole glume takes up pigment. Here also the lower portion is slightly thicker than the top half. This species can be differentiated by the above-mentioned character of the glume from the other three species of this group. *S. dimidiatum* hybridises easily with *S. purpureo-sericeum*. The F_1 was found to have a fully coriaceous lower glume. In length and shape the glumes resemble those of *S. purpureo-sericeum*. The F_2 gave 70 plants with fully coriaceous glumes and 25 with half coriaceous ones like *S. dimidiatum*. The F_2 behaviour was repeated in the F_3 generation, the numbers of the two groups being 33 fully coriaceous and 11 half coriaceous glumes.

It was observed that the length of these spikelets got reduced when the lower glumes became half coriaceous. It seems therefore probable that a single gene is responsible for the half coriaceous nature of the outer glume and the simultaneous shortening of the spikelet. An enlarged picture of the two above-mentioned types of glumes is given in Fig. 3.

S. versicolor.—The word versicolor means change in colour. This refers to the changing of colour of the sessile spikelets from green to blackish purple close on emergence from the boot. This has been called " the black Sudan grass " in the U.S.A.[15] The term black refers probably to the dark colour of the immature spikelets. The name Sudan grass however is a misnomer, for neither is its place of origin the Sudan nor has it any relationship with the typical Sudan grass—*S. sudanense*. This species could be differentiated from *S. purpureo-sericeum* and *S. dimidiatum* by the blackish tint and its fully hairy nature of the glumes. It differs from *S. nitidum* in which the glumes are smaller and the awns shorter.

S. nitidum.—The word nitidum means shining which evidently refers to the shiny nature of the glumes. This species is found in the Western ghats of South India at elevations of 1000 to 7000 ft. It has been found to thrive best in places with a fairly heavy rainfall of 60 to 100 inches annually. On the

hills this plant is probably perennial. It does not however produce any rhizomes. When the top portion of the stem dries up during droughty weather, the bottom portions with the roots remain alive and sprout up with the next rains. These plants did not thrive when brought to the plains. There are two distinct types in *S. nitidum*, one awned and the other awnless. Both these varieties were found to occur in the same habitat. The plants of these species are only half as tall as those of the other three *Para-sorghums* the height being 40–50 cm. as against 100–120 cm. in the others. The tillering capacity also is somewhat less profuse.

Inter-relationship between the Para-sorghums

Stapf while describing *S. dimidiatum* suggested that because of the half coriaceous and half papery nature of the lower glume this species might be a hybrid between *S. versicolor* and a cultivated sorghum like *S. ankolib*, with papery glumes. In Hooker's *Icones Plantarum*[4] it was however pointed out that *S. versicolor* did not occur in the Sudan (the *S. dimidiatum* zone) whereas *S. purpureo-sericeum* was found in that neighbourhood. Therefore it is probable that Stapf might have meant *S. purpureo-sericeum*, when he wrote *S. versicolor*. A hybrid origin of *S. dimidiatum* from a cross between a cultivated sorghum like *S. ankolib* and either *S. purpureo-sericeum* or *S. versicolor* seems to be improbable, because none of the *Para-sorghums* has been observed to cross with any of the *Eu-sorghums*.

The two species *S. purpureo-sericeum* and *S. dimidiatum* are difficult to distinguish from each other but for the half coriaceous glume of the latter. The two species were found to hybridise freely with each other and the half coriaceous glume of *S. dimidiatum* has been found to behave as a monogenic recessive to the fully coriaceous glume of *S. purpureo-sericeum*. The prominently half coriaceous and half papery glume found in *S. dimidiatum* has not so far been met with in any other species of *Sorghum*. Moreover, among the *Eu-sorghums*, in a cross between a fully coriaceous glume and a fully herbaceous glume no intermediate type like the one found in *S. dimidiatum* was obtained either in the F_1 or in the F_2 generation.[16] Hence the Difference between the two species is probably mainly genic.

S. versicolor and *S. nitidum* have a number of characters in common and stand distinct from the other two species of the *Para-sorghum* group. It is interesting to note that *S. purpureo-sericeum* and *S. dimidiatum* have reddish purple glumes while all the other wild sorghums so far examined (including *S. versicolor* and *S. nitidum*) have only blackish purple glumes.

Besides the cross between *S. purpureo-sericeum* and *S. dimidiatum* other crosses also were attempted among these four species. Stray seeds were set, but they all failed to germinate. Further crossing work is in progress with a view to elucidate the genetic inter-relationship amongst them. Attempts to hybridise the *Para-sorghum* with the different members of the *Eu-sorghum* group were not successful.

Economic Aspects

The only publication dealing with the economic aspects of this group is by Kulkarni[17] who described *S. purpureo-sericeum* as a " discovery likely to be useful in the improvement of the grazing areas in the Bombay (Deccan)". The observations made at the Millets Breeding Station, Coimbatore, show that although the non-drying nature of the lower leaves makes it an attractive fodder, the inability of this grass to stand cuttings or even any slight disturbance of the roots makes it uneconomic as a fodder crop under Coimbatore conditions. *S. versicolor* has been tried in America but has not found much favour.

Summary

This paper presents a study of the *Para-sorghums* consisting of four species, namely *S. purpureo-sericeum*, *S. dimidiatum*, *S. versicolor* and *S. nitidum*. The group is characterised by the hairiness of the plants, bearded nodes, very long awns (upto 40 mm.) and five pairs of chromosomes. *S. purpureo-sericeum* and *S. dimidiatum* are very closely related to each other, but for the slightly reduced and half coriaceous glume of the latter. The difference is brought about by a single gene. *S. versicolor* and *S. nitidum* are more closely related to each other than to the other two. The sorghums of this group do not have much value as fodder in South-India, as they are unable to withstand cuttings and succumb to the least disturbance to the root system.

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FIG. 1. Whole plant of *S. purpureo-sericeum*

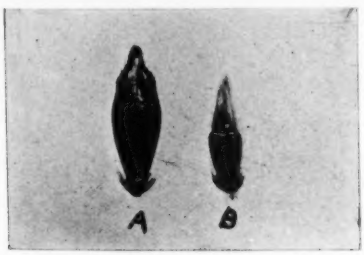

FIG. 2. Glumes—A. *S. purpureo-sericeum*
B. *S. dimidiatum*

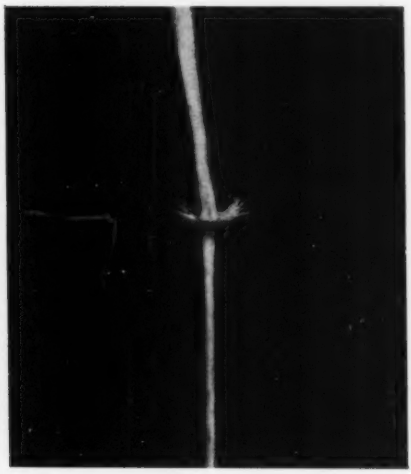

FIG. 3
Bearded node of *S. purpureo-sericeum*

FIG. 4
Spikelets—A. *S. purpureo-sericeum*
B. *S. dimidiatum*
C. *S. versicolor*
D. *S. nitidum*

A HAPLOID PLANT IN *TORIA (BRASSICA CAMPESTRIS* L.)

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1. Introduction

Haploidy in flowering plants was first discovered by Blakeslee *et al.* in 1922. By subjecting pollinated flowers of *Datura stramonium* to low temperature at approximately the time of fertilization, they obtained two haploid plants each of which had 12 somatic chromosomes. Since that time numerous haploids have been discovered and described from a considerable number of different species belonging to widely different families. A detailed discussion of haploidy in flowering plants is contained in a recent paper by Ivanov (1938).

Haploids are usually reduced replicas of their parent plants although in some cases they have been found to show variations in this respect. Thus in *Triticum vulgare* (Namikawa and Kawakami, 1934) and *T. compactum* (Gaines and Aase, 1926) the haploid plants were absolutely identical with the mother plants except in their ill-developed generative organs and high sterility. In *T. dicoccum* and *Tr. persicum* (quoted by Ivanov, 1938) the haploids showed a marked change in their morphological characters. Another feature generally characteristic of the haploids is their high sterility following an absence of conjugation of the chromosomes and random assortment of univalents at meiosis. Consequently haploids produce few progenies. But the occasional

progenies produced by them consist mostly of diploids and rarely of polyploids and trisomics. By far the most interesting progeny of the haploid, from the point of view of the breeder, is the diploid which by reason of its origin is fertile and homozygous. The importance of the production of homozygous diploids from haploids for breeding purposes is emphasised by R. C. C. (1936) and Blakeslee (1939). The latter author commenting on this subject remarks, " Now that we have a means of readily doubling the chromosome number (by the use of colchicine) the great need in our genetic programme is a method whereby we can reduce the number by half ". If we can discover a method by which haploids could be artificially produced at will, then starting with a highly heterozygous plant, such as a fertile species hybrid, we can obtain homozygous lines in two jumps.

As regards the origin of the haploids, they are known to result from generative parthenogenesis or androgenesis either spontaneously with no known cause or under the action of certain agencies which are supposed to stimulate the development of unfertilized egg cells. Among the agents that are known to produce haploids in plants are distant hybridization, and pollination with X-rayed pollen ; in both these cases the male gamete penetrates the embryo-sac but does not fuse with the nucleus of the egg cell. Subjection of pollinated flowers to low temperature at the time of fertilization is also known to induce haploidy. In this case also it is presumed that the male nucleus in the embryo-sac without fusing with the egg nucleus stimulates its development. These methods, however, are neither universally successful in producing haploids nor do they produce haploids in large numbers wherever they are successful.

So the problem of inducing haploidy artificially in plants is still unsolved and is receiving increased attention since the discovery by Blakeslee *et al.* (1937) that the chromosome number in plants can be doubled by the use of colchicine. Connected with this problem is the study of the origin and behaviour of new haploid plants. In this paper, the study of a haploid plant occurring for the first time in *B. campestris* is reported.

2. *Previous Work in* Brassica

In the genus ***Brassica*** haploids have been reported only in the species *B. napella* (Morinaga and Fukushima, 1933). They were highly sterile indivi, duals occurring rather frequently in rape fields. According to these authors, " Half a day's excursion through the rape field, when the flowers are passing away, will suffice to find dozens of such haploid individuals." The cause of their occurence in such large numbers is, however, not known. The leaves and flowers of the haploid were obviously smaller than those of the diploid

but the height, though generally lower than that of the diploid, ultimately surpassed the latter on account of continuous development of inflorescences. In the root tip cells, 19 chromosomes were found in the haploid compared to 38 in the diploid. The meiosis in the haploid was irregular following the production of 0–6 or 7 bivalents at division I. The formation of so many bivalents in the haploid is explained on the basis that *B. napella* is an allopolyploid species (Morinaga, 1929) with two duplicated genomes. Most of the pollen grains formed in the haploids were small and shrivelled while some were large and perfect.

At the Imperial Agricultural Research Institute, where cytogenetic investigations on the oleiferous *Brassicæ* are in progress, a spontaneously occurring haploid plant was discovered in one of the cultures of *rai*, *B. juncea* (Ramanujam, unpublished). This species with $2n = 36$ chromosomes, like *B. napella*, is an allopolyploid species (U, 1935) and as a consequence, the haploid showed varying numbers of bivalents at meiosis.

The occurrence of haploid plants in elementary species of *Brassica* with $n = 8$, 9 and 10 chromosomes is so far unknown and this paper reports the first occurrence of a haploid plant in such an elementary species, *viz.*, *B. campestris* with $n = 10$ chromosomes.

3. Origin and Description of the Haploid

During the year 1940–41, in one of the cultures of *toria* grown from an open-pollinated plant, a dwarfish looking plant with smaller leaves and flowers compared to other plants (Pl. II, Figs. 1–4) in the line was noticed. It flowered almost at the same time as the other plants and set a fair amount of seed under conditions of open-pollination. As the plant was in every respect a reduced replica of the other plants, it was suspected to be a haploid. An examination of its pollen was undertaken and it was found that they were fairly fertile with only about 40 per cent. empty and shrivelled grains. This was rather unusual, as in most of the haploids previously described the sterility of pollen was very high as much as 90 per cent. or more in some cases. The epidermal cells of the leaf in the haploid were examined and the size of stoma guard cells was compared with that of the normal plants. Although many of the stomata of this abnormal plant were considerably smaller than those of the normal plants, a fair number of them was similar in size to those of the latter. An examination of the chromosomes definitely showed that this abnormal plant was a haploid. The plant was selfed and crossed reciprocally with diploid plants in the row, while a few branches were left for open-pollination. No seeds were obtained from selfed flowers, presumably owing to the self-sterile nature of the species, but a good amount of seed was

obtained from open- and cross-pollination. Unlike the haploid plants of *B. napella* (*loc. cit.*), this haploid did not exhibit a continuous development of inflorescences but died with the rest of its sister plants in spite of careful attention to perpetuate it by cultural means. It is probable that the fairly high degree of seed setting in the present haploid did not promote its continuous vegetative development.

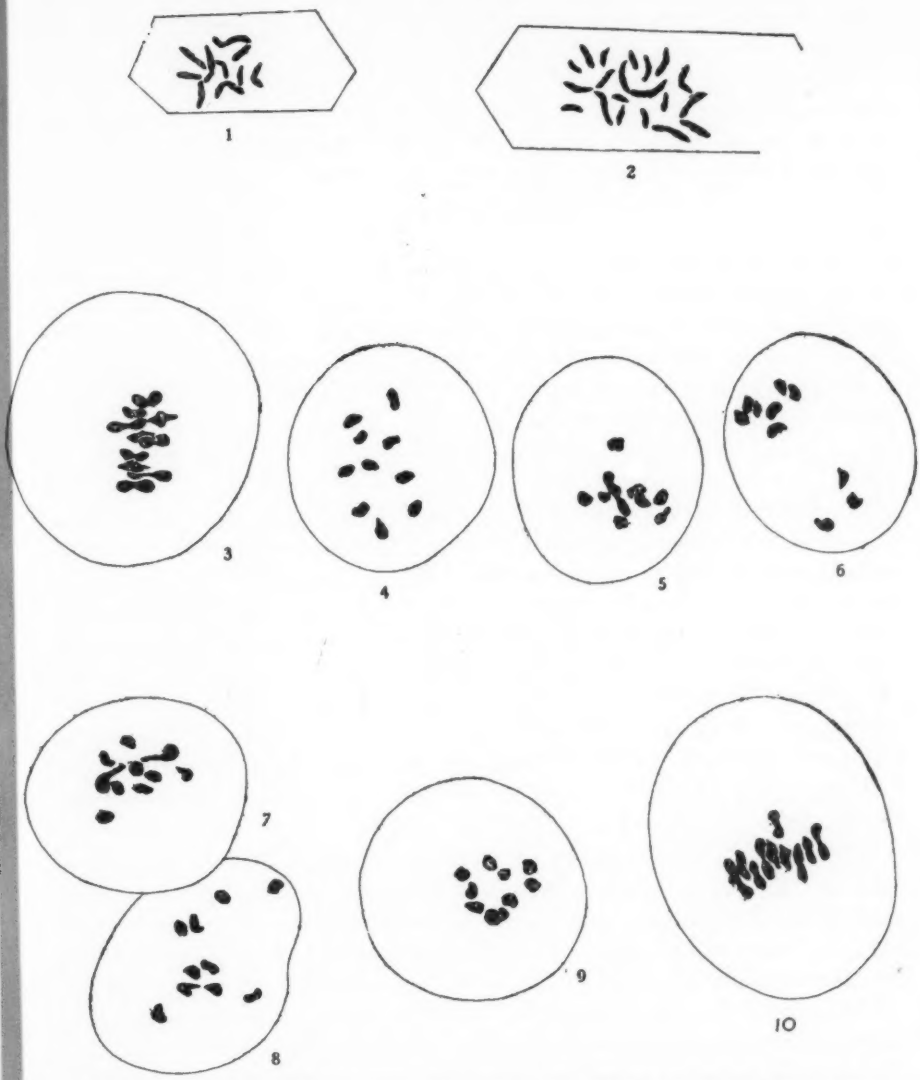
4. Cytological Technique

The somatic chromosomes were studied in the meristematic cells of young leaves smeared and stained with aceto-carmine adopting Baldwin's technique (1939). Meiosis in pollen mother cells was studied in permanent aceto-carmine smears (McClintock, 1929). Drawings were made with the aid of a camera lucida at bench level using a 2 mm. apochromatic objective and 15X and 20X compensating oculars.

5. Cytology of the Haploid

The somatic chromosome numbers of the haploid and the diploid were counted in the meristematic cells of young leaves and found to be 10 (Text-Fig. 1, and Pl. III, Fig. 4) in the case of the former and 20 (Text-Fig. 2 and Pl. III, Fig. 3) in the case of the latter. In the haploid smears examined, a few big cells with double the number of chromosomes, *i.e.*, 20, were found intermingled with normal cells with 10 chromosomes. The occurrence of bigger stoma guard cells in the epidermis of the haploid, which was mentioned earlier, is perhaps due to the presence of such diploid cells. Diploid cells in haploid tissue have been recorded in several plants (Ivanov, 1938). In the diploid material examined in the present case, however, no duplicated cells were observed.

The meiosis in the haploid was followed from diakinesis to pollen formation and compared with that in the diploid. In the pollen mother cells of the diploid 10 bivalents were observed at diakinesis and metaphase I (Text-Fig. 3). Subsequent stages of the meiosis were carried out regularly and normal tetrads were formed, which gave rise to fertile pollen. In the haploid, at the stage of diakinesis there could be clearly seen in each pollen mother cell 10 univalents scattered about the periphery of the nucleus. At a later stage, in which the distinction between metaphase I and anaphase I was obscured, the ten univalents were found distributed on a faintly differentiated spindle. Text-Figs. 4, 6 and 9 show the disposition of univalents at this stage. Bivalents were rarely met with except in a few cells in each of which one rod-shaped bivalent was noticed (Text-Figs. 5, 7 and 8). In such cells the bivalents soon separated out into their components. The later behaviour of the univalents



was found to take one of two courses. In a few cells the scattered univalents were seen to segregate into two groups (Text-Fig. 6) following a random distribution, while in a large majority of cases, they seemed to collect together and form an interphase nucleus containing all the ten chromosomes. In the former case, where there was random segregation of chromosomes, the division II proceeded fairly normally and gave rise to tetrads with unequal

pairs of spores. In the latter case, where the segregation was suppressed, the division II yielded dyads with two equal spores. This kind of suppression of the first division and the formation of dyads were noticed to a large degree in haploid *Matthiola* (Lesley and Frost, 1928) and to a lesser degree in haploids of other species. In the case of *Matthiola* (*loc. cit.*), however, the univalents at metaphase were long and bent, simulating the somatic chromosomes. Another interesting feature of meiosis in the haploid was the occurrence of diploid pollen mother cells. These were bigger than the haploid cells and were found usually scattered in the anther locules. They showed normal pairing of chromosomes into ten bivalents at metaphase I (Text-Fig. 10) and produced normal tetrads following a regular meiosis. Pl. III, Figs. 1 and 2 are photomicrographs showing pollen mother cells of the haploid plant in various stages of meiosis. In Pl. III, Fig. 2, two diploid cells, which are bigger than the surrounding haploid cells, may be seen at side view of metaphase I with ten bivalents in each. The occurrence of pollen mother cells with double the number of chromosomes has been recorded in several plants. In *Oryza officinalis* (Ramanujam, 1938) the occurrence of big pollen mother cells intermingled with normal ones was recorded. Tetraploid pollen mother cells were also noticed in the anther locules of diploid *œnothera* (Cleland, 1929) and *Brassica* (Fukushima, 1931), etc. These polyploid cells may arise by the duplication of chromosomes in the last premeiotic division or earlier during the formation of the sporogenous tissue ; in the former case isolated big cells and in the latter, groups of them will be formed. In the present case, the diploid cells were found mostly scattered and, therefore, may have resulted from sporadic autopolyploidy in cells at the last premeiotic division. The occurrence of diploid pollen mother cells considered in relation to the presence of scattered diploid cells in the epidermis and meristematic cells of leaves would appear to indicate that the haploid has a tendency to produce doubled cells throughout its ontogeny. How exactly this doubling is brought about is not known. One other fact worth mentioning in this connection concerns the pairing behaviour of the chromosomes in the diploid pollen mother cells of the haploid. While in the case of *Brassica* (*loc. cit.*) the groups of tetraploid cells in the diploid, which according to Fukushima arose from initial archesporial cells, did not show any appreciable tetravalent formation which would be expected in such autopolyploid cells, in the case of the diploid cells in the present case, the chromosomes were perfectly paired into bivalents according to expectation. It may be mentioned in this connection that in autotetraploid Kale (Howard, 1939) and autotetrapolid *toria*, *B. campestris* (Ramanujam, unpublished) varying numbers of quadrivalents were regularly found at meiosis. This difference in the pairing properties

of homologous chromosomes only emphasises the complicated nature of the phenomenon of chromosome conjugation (Ramanujam, 1937).

An estimation of sterility of pollen in the haploid was made by counting the perfect and well filled (fertile) and the shrivelled and empty (sterile) grains in several anthers. A count in five different samples gave 617 fertile and 440 sterile grains which works out 41·6 per cent. sterility in the haploid compared to practically no sterility in the diploid. A similar count of tetrads and dyads was made from several anthers and the figures obtained were 472 dyads and 174 tetrads. Assuming that the tetrads would give rise to sterile pollen on the expectation that they would contain unbalanced chromosome numbers and the dyads to fertile pollen as they would have the haploid complement of chromosomes, 944 fertile and 696 sterile pollen grains would be obtained from the sporads examined. These figures work out to 42·4 per cent. sterility in the haploid which is more or less in agreement with the result obtained by directly counting the pollen grains. It may be pointed out that the slight increase in sterility obtained from sporad counting may be due to the fact that some of the tetrads which would be obtained from diploid pollen mother cells and which would yield fertile pollen are included in the sterile class. Pollen grains of the diploid and the haploid are shown in Pl. III, Figs. 5 and 6. The fertile pollen grains of the haploid were similar in size to those of the diploid. The fairly good setting of seed obtained in reciprocal crosses between the haploid and the diploid shows that many of the pollen grains and egg cells are functional in the haploid. Selfing the haploid did not produce any seed presumably due to self-sterility, which is characteristic of the species.

6. *Discussion*

The importance of the production of haploid plants for breeding purposes has already been referred to. Although it is obvious that the egg cells of many of the flowering plants are capable of developing into haploid plants, the question as to what precisely stimulates their parthenogenetic development is far from solved. Several investigators have endeavoured to obtain haploid plants by artificial means with very little success. Ivanov (1938) tried several methods for stimulating egg cells of *Nicotiana* to develop into haploids and succeeded in getting only a few by pollination of plants with X-rayed pollen. He also tried, among other things, pollinating *Nicotiana* plants with pollen from plants belonging to the other genera of the Solanaceæ without any success. In the genus *Brassica*, a number of interspecific and a few intergeneric crosses were made and a good amount of crossed seeds was obtained by the author of the present paper ; these seeds in many cases, however, gave rise to plants resembling the mother parents. Noguchi (1928),

Ali Mohammad and Sikka (1940) also reported the occurrence of maternal offspring in the progenies of interspecific crosses in *Brassica*. The maternal offspring in all these cases may have developed from the vegetative tissue of the mother plant or have arisen due to induced parthenogenesis with ensuing diploidy. East (1930) working with *Fragaria* showed that, in some cases at least, the latter process was involved and pointed out its significance to practical breeding. If a reasonably large percentage of parthenogenetic embryos can be forced to develop into diploids by interspecific hybridization or other means, the results would be of great value for breeding purposes. Starting with a highly heterozygous plant, such as a species hybrid, we could directly obtain from it homozygous lines with different combinations of the characters of the two parents. This method of obtaining homozygotes eliminates the necessity of obtaining haploids and then doubling the chromosomes, which was indicated earlier in the paper; the doubling in this case takes place earlier in the developing haploid embryo without the influence of any external agency. Experiments are in progress in the Institute to determine the mode of origin of diploid offspring in interspecific and intergeneric crosses of *Brassica* with a view to the practical application of the knowledge for breeding superior varieties.

The haploid *Brassica* described in this paper resembles most of the other haploids in being a reduced replica of the parent plant. Its tendency to double the chromosomes in the somatic and sporogenous cells, however, is a noteworthy feature which accounts for its high fertility. The study of the progeny of this plant will be taken up next year. Any diploids that may be obtained in the progeny will not be homozygous as selfed seeds were not obtained.

7. *Summary*

A spontaneously occurring haploid plant in one of the open-pollinated cultures of *toria* is described for the first time.

The haploid was a reduced replica of the diploid plant with smaller leaves and flowers. It flowered at the same time as the diploid and after setting a good amount of seed under conditions of open-pollination died with the rest of its sister plants.

The somatic chromosome number of the haploid was determined in meristematic cells of young leaves as 10 and that of the diploid as 20. A few cells with the diploid number of chromosomes were found scattered in the leaf tissue of the haploid.

At meiosis, the haploid showed 10 univalents from diakinesis to anaphase I in each pollen mother cell. Occasionally, however, a loosely paired

bivalent was noticed in some cells. At division I the univalents either segregated at random into two groups or collected to form an interphase nucleus. Division II in the former case produced tetrads of unequal spores, and in the latter dyads of equal spores.

A few pollen mother cells with the diploid number of chromosomes were also noticed in the haploid; these were seen to undergo normal meiosis producing tetrads with equal spores.

As a result of dyad formation in a large number of haploid pollen mother cells and tetrad formation in the few diploid pollen mother cells, the pollen fertility of the haploid was found to be as high as about 60 per cent. Seed-setting in the haploid was also fairly high under conditions of open and cross-pollination.

The possible utilisation of haploidy in practical breeding is discussed.

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EXPLANATION OF PLATES AND TEXT-FIGURES

PLATE II

FIGS. 1–2.—Photographs of diploid and haploid *toria*, respectively.

FIGS. 3–4.—Photographs of flowers of the diploid and haploid *toria*, respectively.

PLATE III

FIGS. 1–2.—Photomicrographs showing pollen mother cells of the haploid plant in various stages of meiosis.

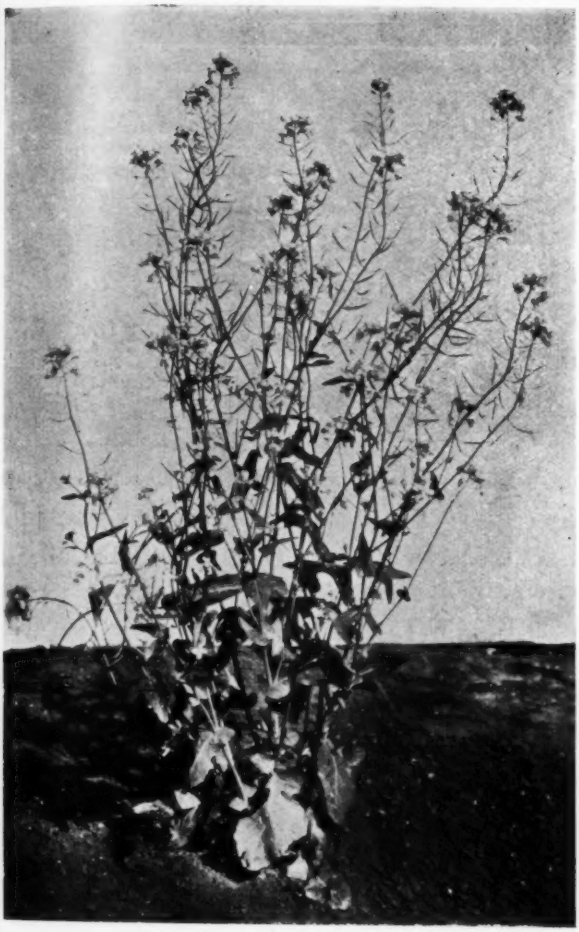
(Note in Fig. 2 two diploid cells, bigger than the surrounding haploid cells, in side view of metaphase I with ten bivalents.)

FIGS. 3–4.—Photomicrographs of somatic chromosomes in meristematic cells of young leaf of the diploid and the haploid, respectively.

FIGS. 5–6.—Photomicrographs of pollen grains of the diploid and the haploid, respectively.

TEXT-FIGS. 1–10

FIGS. 1–2. Somatic chromosomes of the haploid and the diploid, respectively, in the meristematic cells of young leaves. FIG. 3. Pollen meiosis in the diploid, showing side view of metaphase I. FIGS. 4, 6 and 9. Pollen meiosis in the haploid, showing the disposition of univalents at metaphase I and anaphase I. FIGS. 5, 7 and 8. Pollen meiosis in the haploid, showing one rod shaped bivalent in each cell. FIG. 10. A diploid cell in the haploid at metaphase I (side view with ten bivalents.)



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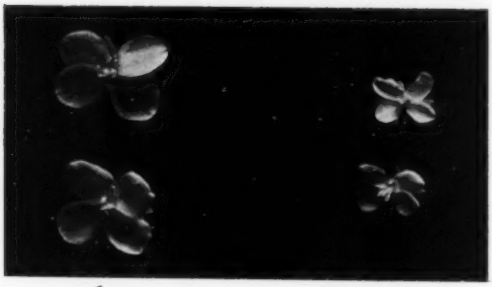

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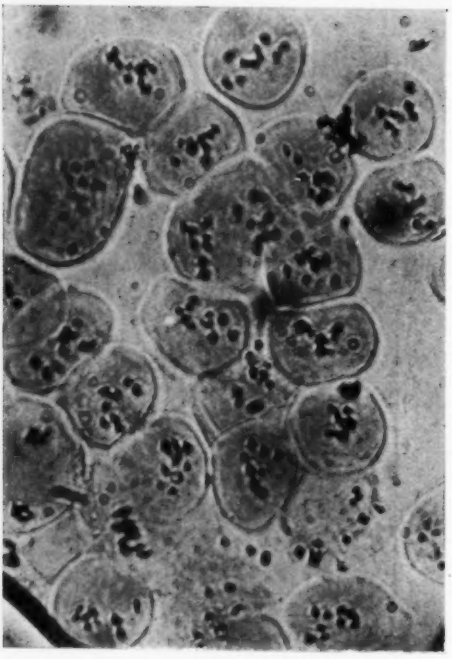

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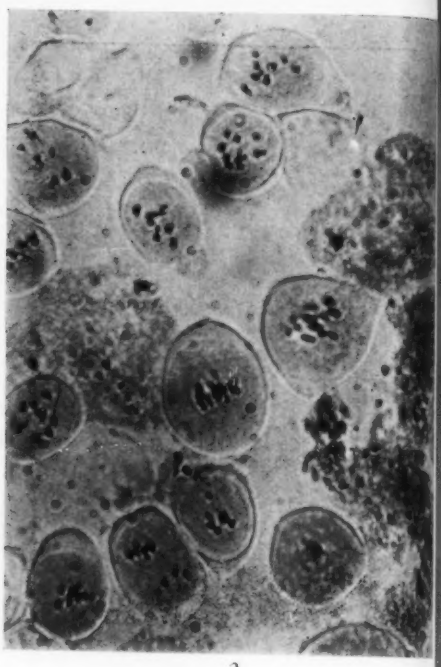

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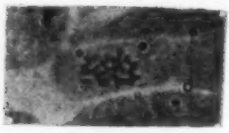

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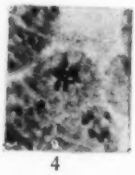

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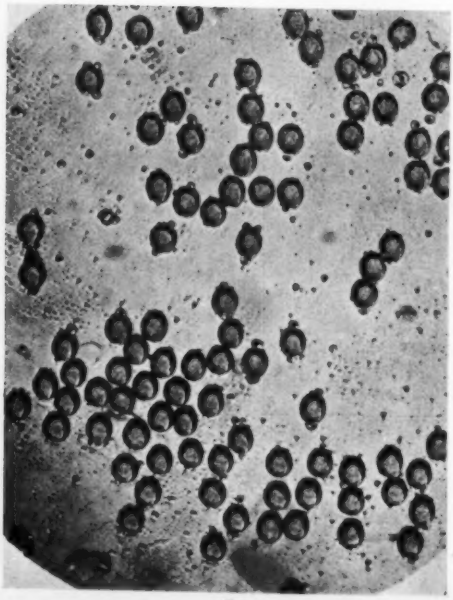

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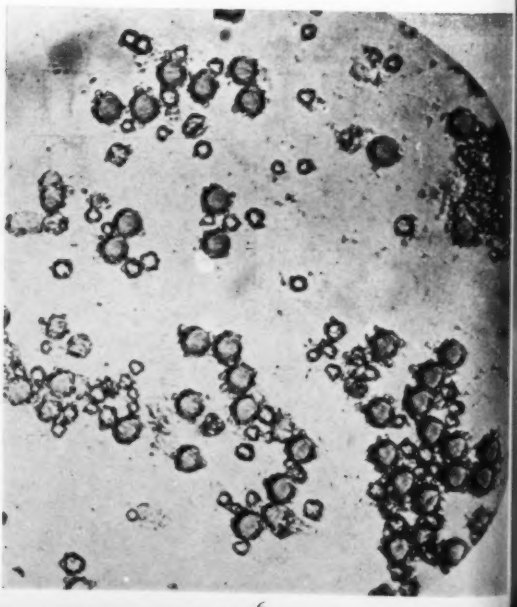

6

CYTOGENETICAL STUDIES IN NICOTIANA

Part II. Morphological Features of *Nicotiana glutinosa* and the Hybrid between *Nicotiana glutinosa* and *N. tabacum*

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1. Introduction

Studies of embryo-sac formation and embryo development in Solanaceæ have been extensively made. Various genera have been investigated and recently Bhaduri (1935) has made a detailed study of the development of female gametophytes in many of the tropical genera of this family.

The genus *Nicotiana* has been the subject of morphological investigations even as early as 1870 and is probably the first genus in this family to be investigated in this light. According to Schnarf (1931), Hanstein (1870) studied the embryo development in this genus. Chatin (1874) studied the development of ovule and pollen grains in *Nicotiana*. He has described the ovules of *Nicotiana rustica* and *N. tabacum* as anatropous. Elfing (1879) studied the pollen development in *Nicotiana*. It was Guignard (1882) who first worked out in detail the development of embryo-sac and embryo in *Nicotiana tabacum* and found that the development was of the normal type. Species other than *tabacum* were not studied in detail until Ventura (1930)

gave an account of the embryo development in *Nicotiana sylvestris*. Recently Bhaduri (1935) has described the development of female gametophtye in *Nicotiana plumbaginifolia*.

Morphological investigations have not been carried out in *Nicotiana glutinosa* as far as the available literature could show. The present communication will deal with the development of anther, embryo-sac and embryo in *Nicotiana glutinosa*. The arrested development of the megaspore in the *glutinosa* × *tabacum* hybrid and the differences in details of embryo-sac development between the *glutinosa* and the *tabacum* parents have also been described.

2. Description of the parents and the hybrid (Pl. IV)

(*a*) *Description of the parents :*

The *tabacum* parent is a cultivated variety of chewing tobacco. All the Indian varieties which are large leaved have been classified by Howard and Howard (1910). The Indian varieties belong to the variety *macrophylla* according to the classification of Comes (1899, 1905).

The plant grows to a height of about 4½ to 5 feet (Pl. IV B). The growth of the stems, which are stout, is erect and not spreading, and axillary branches from the basal leaves arise only late in the life of the plant. The stem branches irregularly at the apex. The leaves are sessile and their bases clasp round the stem. This auriculate leaf base is characteristic of this parent. The leaf-base gradually broadens into the lamina which is one foot broad when very big and 1½ to 2 feet long. At the apex the leaf is considerably small and at the inflorescense region, is reduced to the size of scale leaves from the axils of which peduncles arise. The leaf tips are acute and the midrib is very stout and strong and the venation of the leaf is pinnate, and coarsely reticulate. The inflorescense is a compound cyme. The flowers have funnel-shaped corolla the tube of which is white in colour while the lobes are pink. The corolla is actinomorphic. The stamens are of varying lengths, and are epipetalous. The dehiscence of anthers is towards the centre of the flower. Capsules are big and are ovate in shape.

The glutinosa parent.—The *glutinosa* species differ very much from tabacum in external appearance. The former is more fragile. The stem of *glutinosa* is more slender and at the apical portion it is very weak. Branching occurs to a very limited extent.

Leaves are distinctly petiolate. They are cordate in shape with pinnately reticulate venation. The size of the leaves is very small, having a maximum length of nine inches and breadth of six inches.

Inflorescences arise mostly terminally and are distinctly racemose. Long peduncles bear older flowers at the base and younger at the apex. The flower shows marked disparity with that of *tabacum*. Instead of the straight funnel-shaped corolla there is a strong tendency towards formation of bilabiate corolla. The colour of the corolla is deep orange. The anthers are bigger and longer than in the case of the tabacum parent. The anthers and style remain in a position very much similar to that found in bilabiate flowers of other families. They lie adhering to the upper lip-like lobe of the corolla.

The capsules are smaller and are conical in shape.

(*b*) *The hybrid :*

The origin of the hybrid will be described in detail later. The hybrid was obtained from crossing experiments conducted at the botanical garden at Annamalainagar. The hybrid for all external appearances resembles the *tabacum* parent. The leaves are as big as the leaves of *tabacum* and have pinnate venation and auriculate leaf bases, though they are not as broad at the basal portion as the leaves of the *tabacum* parent are.

The inflorescence shows a marked tendency towards the racemose form of the *glutinosa*. The flowers are carmine in colour as in *tabacum* but are slightly zygomorphic. Anthers in this case are shrivelled and unhealthy.

No capsules were obtained as the flowers fall off after blossoming. Early during the growth of the flower an abscission layer is formed at the base of the pedicel which causes the flower to be shed. Such phenomena have been reported by Goodspeed (1913 *b*) in the F_1 of *Nicotiana sylvestris* × *N. tabacum*. Unlike those hybrids which were partially fertile these were completely sterile. The sterility in this case is due to defective pollen and arrested development of the female gametophyte.

3. The technique of crossing

The parents *Nicotiana tabacum* var. marcophylla (Coimbatore) and *N. glutinosa* were got from the agricultural research station at Coimbatore. These parents were grown in the University botanical garden during January 1940, when crossing experiments were done.

The largeness of the flowers, the good amount of pollen from freshly dehisced anthers and the ready viability of the pollen of one parent on the stigma of the other, facilitated the success of the crosses. The hybridization involves three processes, one immediately following the other. These were emasculation, pollination and protection of pollinated flowers.

Emasculation.—Emasculation is the removal of anthers from the flowers which are to be pollinated, in order to destroy all chances of self-pollination. The preliminary step taken was to study the growth of the anther, style, and the bud on the whole, in order to determine the correct stage for emasculation. In very young buds the anther was found much below the stigma. But later the anthers come to occupy a position just above the style. At this time the anther did not dehisce. The stigma was receptive just after this period (*i.e.*, protogynous) and this was found to be the correct stage for emasculation.

Before emasculation was done, care was taken to see that no anther had dehisced. All the buds except those to be pollinated were removed from the inflorescence axis.

Emasculation is easily performed in this case. The corolla is slightly split just above the calyx right up to the lobes with a needle. If the split corolla is pushed back with the fingers, the anthers and the stigma are exposed for operation. The anthers are now cut in the region of the filament with the help of a pair of pointed scissors and removed away, lest they should stick to the branch and contaminate the pollinated flowers.

Pollination.—Pollination was done immediately after emasculation, though the stigma was not receptive at that time. This latter fact does not affect the success of the crosses, because in dry weather as prevailed in these months, the pollen of *Nicotiana* species was viable for a number of days. Anthers which had just dehisced could be obtained in plenty and these were brushed against the stigma so that large numbers of pollen grains were deposited on the stigmatic surface.

The evening time was found suitable for these operations. If it was done in the mornings the injury caused to the flower is aggravated by the hot weather which prevails during the day. So crosses effected in the mid-day or in the morning were not so successful as those effected in the evening. Though reciprocal crosses succeeded, the more successful cross was that with *tabacum* as the female parent.

Protection of flowers.—After emasculation and pollination, the operated flowers were protected from contamination by foreign pollen. This was done by bagging the flowers that had been pollinated. Cellophane bags were employed for the purpose and they were tied round the stem by thin copper wires at their bases. Labels were attached containing information about the crosses.

One month elapsed before the pollinated flowers would give mature capsules. The capsules and the seeds did not show any sign of hybridity.

Care was taken to see that the capsules did not dehisce. When the capsules had assumed a brown colour all over, including the persistent calyx portion, they were harvested. Then they were dried in special drying shelves.

The seeds were sown four months after the harvest at fortnightly intervals. The percentage of germination increased with the time between harvesting and sowing.

4. Meterials and methods

The ovaries of the parents and the hybrids were cut into small pieces to facilitate proper fixation and were fixed in formalin-acetic-alcohol, and corrossive sublimate fixative. For embryo stages ovules were fixed separately. The materials were imbedded in paraffin with chloroform as paraffin solvent. Sections were cut at thicknesses varying from 6–15 microns and stained in Haidenhain's iron-alum Hæmotoxylin.

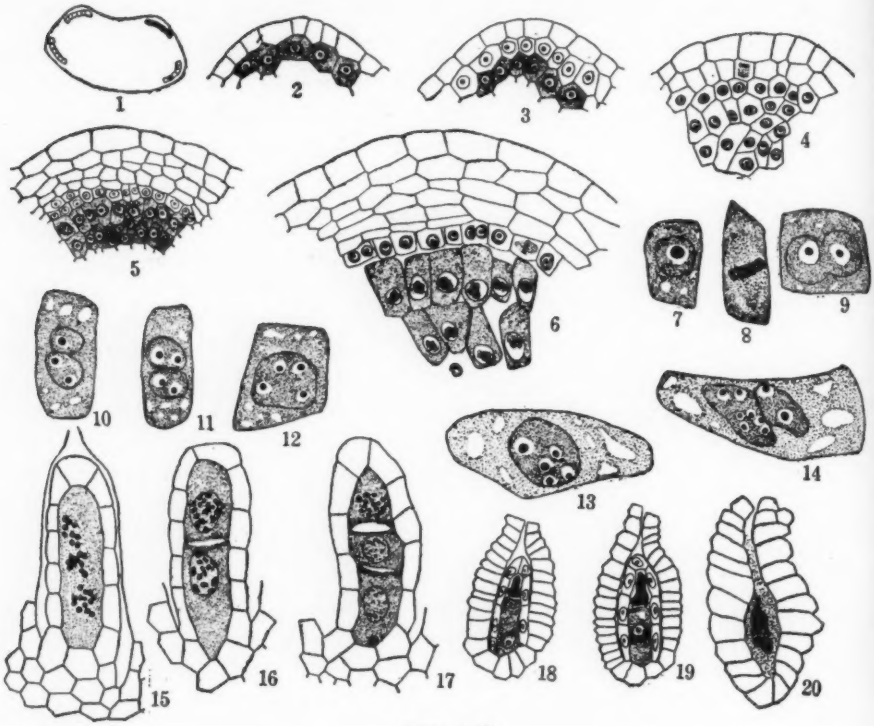
5. Development of anther in *N. glutinosa*

The primary archesporium is differentiated as four bands of hypodermal cells, one in each lobe, very soon after the anther appears to be four lobed (Fig. 1). Each band consists of 5–6 cells in cross-section (Fig. 2). Such bands of archesporia instead of a single archesporium have been met with in *Digera arvensis* (Joshi and Rao, 1934) and *Portuluca oleracea* (Cooper, 1935).

The archesporial cells undergo periclinal divisions giving rise to a layer of primary wall cells outside and a layer of primary sporogenous cells inside (Fig. 3). Through the division of the primary wall cells, four to five layers of wall cells are formed (Fig. 4), of which the innermost functions as the tapetum (Figs. 5 and 6).

The tapetal cells enlarge with formation of vacuoles (Fig. 7). The nuclei of the tapetal cells divide mitotically giving rise to bi-nucleate tapetal cells, at the time the microsporemother-cells are about to enter upon the meiotic stages (Fig. 8). This gives us additional evidence to disprove the possibility of an amitotic division, by which the tapetal nuclei are held by some authors to multiply.

The behaviour of the tapetal nuclei after this is rather irregular. Sometimes the two nuclei fuse to form a single large binucleolated nucleus (Fig. 9). Figs. 10–14 show the nuclei in the various stages of fusion. Figure 14 represents a case where two nuclei are seen, one with 7 and the other with 2 nucleoli. All these stages very often appear side by side in the same tapetum. A similar behaviour of tapetal nuclei has been recorded



FIGS. 1–20.

FIGS. 1–14.—*Microsporangium development in Nicotiana glutinosa.* FIG. 1. Section of a very young anther showing the hypodermal bands of archesporium. × 150. FIG. 2. A band of primary archesporial cells. × 750. FIG. 3. Formation of one layer of primary parietal cells outside and one layer of sporogenous cells inside, due to the periclinal division of the archesporia cells. × 750. FIG. 4. Division of the primary wall cells. × 750. FIG. 5. Showing 4–5 layers of wall cells, with uninucleate tapetal cells. × 500. FIG. 6. The same at a later stage showing mitotic division of tapetal cells and bi-nucleate tapetal cells. × 750. FIG. 7. A uni-nucleate tapetal cell. × 2200. FIG. 8. Mitotic division in the tapetal cell. × 2200. FIG. 9. Bi-nucleate tapetal cell, the two nuclei in the process of fusion. × 2200. FIG. 10. Binucleate cell one of the nucleus having two nucleoli, probably arisen from fusion of two nuclei. × 1500 FIG. 11. Bi-nucleate cell both the two nuclei having 2 nucleoli. × 1500. FIG. 12. A 4-nucleolate nucleus in the tapetal cell. × 1500. FIG. 13. A single 5 nucleolate nucleus in the tapetal cell. × 1500. FIG. 14. Two nuclei in the tapetal cell one having 7 nucleoli and the other only two. × 1500. FIGS. 15–20. *N. glutinosa* × *N. tabacum* F_1 *hybrid*. FIG. 15. Mega-sporemother-cell of the hybrid in division showing $28_I + 4_{II}$ configuration and the scattered arrangement of chromosomes. × 1500. FIG. 16. Dyad stage. × 1500. FIG. 17. Division in the micropylar dyad, while division has been completed in the chalazal dyad. × 1500. Fig. 18. Degeneration of the micropylar dyad without completion of division. × 750. FIG. 19. Degeneration of all cells except the chalazal megaspore; shows the formation of tapetum and the persistent nucellar layer. × 750. FIG. 20. Degeneration of the megaspore. The nucellar cells have degenerated and the tapetal cells have grown large in size. × 750.

in *Gynandropsis pentaphylla* (Raghavan, 1938) and *Ilysanthes parviflora* (Raghavan and Srinivasan, V. K., 1941).

The sporogenous cells after this round off and enter into the meiotic stages. The meiosis and pollen formation in this plant has been described in detail in a previous paper (Raghavan and Srinivasan, A. R., 1941).

6. Megasporogenesis and embryo formation in *N. glutinosa*

The ovary is typically solanaceous with two axile placentæ bearing numerous ovules. The ovules are at first seen to arise as straight outgrowths from the placenta. At this time, the archesporium of the megaspore is differentiated from the hypodermal layer of the ovular out-growth (Fig. 21). A little later, however, the ovule bends towards one side, and the integumental tissue is now differentiated from the nucellar tissue. The integument grows rapidly and when the megasporemother-cell is formed, there is a single massive integument. In Fig. 22 megasporemother-cell is long and its nucleus has entered into the meiotic prophase stages. The megasporemother-cell is surrounded by the single nucellar layer on all the three sides except the chalazal end. This nucellar layer is short-lived, for it is soon caught between the growing megaspore and the integument and is finally crushed and degenerates (Fig. 25).

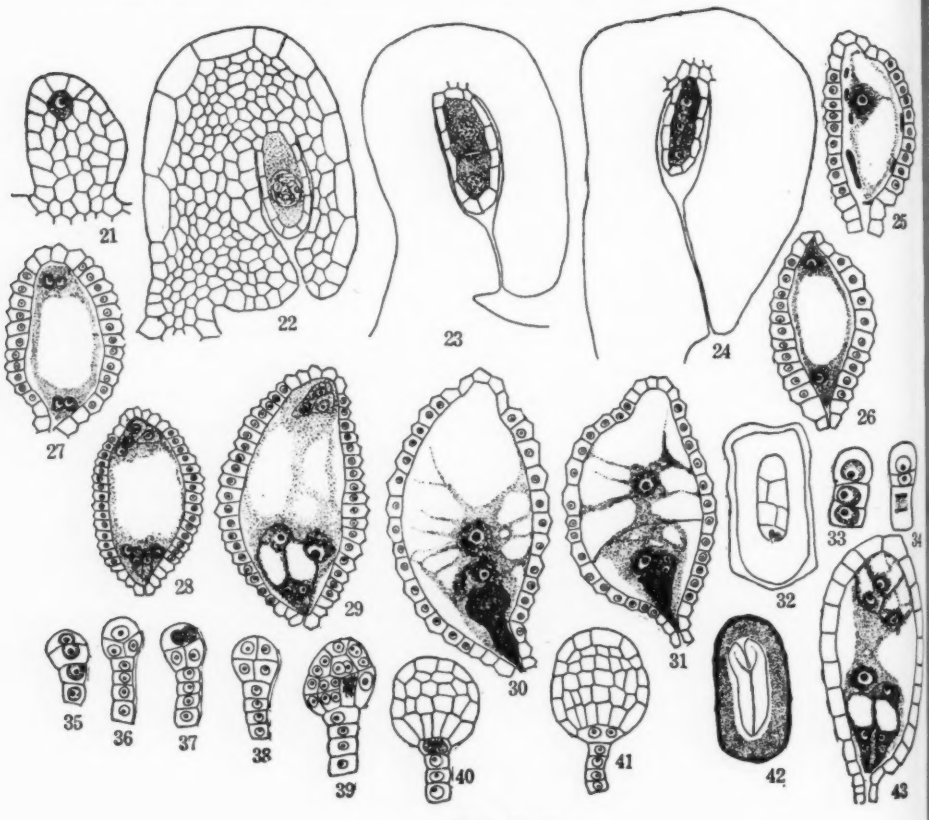
Due to the breakdown of this nucellar layer, the embryo-sac is brought in contact with the integumental tissue. At this time the innermost layer of the integument, which is in contact with the embryo-sac, is differentiated from the rest by elongating radially and appearing as a row or band of cells on either side of the embryo-sac (Fig. 25). This layer of cells is the tapetum and has a nutritive function. Cooper (1931) has recorded a similar method of origin of tapetum in *Lycopersicum esculentum*. In this case the tapetum does not surround the whole embryo-sac. At the chalazal and micropylar ends only ordinary cells are found (Figs. 28 and 29). Such cases have been met with in *Dopatrium* (Srinivasan, 1940) and in species of *Vandellia* (Krishna Iyengar, 1939). Schmid (1906) has found that the tapetum might cover the whole embryo-sac in certain cases. The tapetal cells are more or less rectangular in shape, with their long axis lying perpendicular to the embryo-sac. They contain dense cytoplasm and a single nucleus each. With the growth of the tapetal cells vacuoles are formed. Bi-nucleate tapetal cells have been reported in *Solanum melongena* (Bhaduri, 1932) and *Orobanche ægyptiaca* (Srivastava, 1939). Distinct tapetal tissues have been met with in *Labiateæ* (Billings, 1909) and in *Lobeliaceæ* (Kausik, 1938) also.

The megasporemother-cell is rich in cytoplasm and its nucleus undergoes the two meiotic divisions giving rise to the dyad and tetrad (Figs. 23 and

24). The tetrad is linear and the chalazal cell develops into the uninucleate embryo-sac while the other three cells degenerate.

In the uninucleate embryo-sac, two big vacuoles are formed (Fig. 25). The two, four and eight-nucleate embryo-sacs are formed by the division of the nucleus (Figs. 26, 27 and 28). The mature embryo-sac is thus formed by three successive divisions of the megaspore nucleus. This conforms to the normal monosporic type of embryo-sac development (Maheshwari, 1937).

Of the 8 nuclei, one from each end migrates towards the centre (Fig. 28). The egg-apparatus is organised and the polar-nuclei fuse to give rise to the



FIGS. 21–43.

FIGS. 21–42. *Nicotiana glutinosa*.—FIG. 21. Archesporium. FIG. 22. Megaspore-mother cell, with the massive integument and a single nucellar layer. Fig. 23. Dyad stage. FIG. 24. Linear tetrad. FIGS. 25, 26 and 27. Uni-, bi- and 4-nucleate embryo-sacs. The apetal layer surrounds the embryo-sacs. FIG. 28. 8-nucleate embryo-sac, the polar nuclei

starting towards the centre. FIG. 29. Mature embryo-sac showing the egg apparatus, the secondary nucleus and the ephemeral antipodals. FIG. 30. Zygote and endosperm nucleus. FIG. 31. After the first division of the endosperm nucleus. FIG. 32. 6-celled endosperm, showing that 4 periclinal and 2 anticlinal divisions have taken place. FIG. 33. 3-celled pro-embryo. FIG. 34. The same with the lowermost cell undergoing division. FIG. 35. 4-celled pro-embryo, the upper two cells bigger than the lower two. FIG. 36. Quadrant. FIGS. 37–38. Octant. FIG. 39. Division of the octant cells, in most of which wall formation has been delayed resulting in cells with two nuclei. FIG. 40. Hypophysis in division. FIG. 41. The upper daughter cell of the hypophysis divided but wall formation has been delayed. Fig. 42. Mature seed. All the figures except 32 and 42 are of magnification. × 750. FIG. 32 is × 150. FIG. 42. is × 75. FIG. 43. *Nicotiana tabacum*.—FIG. 43. Mature embryo-sac of tabacum showing the absence of distinct tapetal tissue. The antipodals are large with big prominent nuclei. × 750.

secondary nucleus (Fig. 29). The antipodals in this case are small and are ephemeral. In most of the sections antipodals could not be observed. Bhaduri (1935) observes that the outline of the antipodal cells remain long after fertilization in *Nicotiana plumbaginifolia*. Such extremely ephemeral antipodals as are met with in the present case were observed in *Dentella repens* (Raghavan and Rangaswami, 1941).

After fertilization the endosperm nucleus migrates to a more central position (Fig. 30). The endosperm is cellular. The first division of the endosperm is transverse (Fig. 31). The first two divisions of the endosperm are periclinal and anticlinal divisions begin only after these periclinal divisions are completed (Fig. 32). The zygote undergoes transverse divisions giving rise to the 3 celled pro-embryo (Fig. 33). The basal cell undergoes division and a 4-celled pro-embryo is formed in which the two apical cells are larger than the other two (Figs. 34 and 35). The quadrant is formed by the divisions of the cell next to the apical cell (Fig. 36). The apical cell also divides giving rise to the octant (Figs. 37 and 38). At this period the suspensor consists of 4 cells, that nearest to the embryo forming the hypophysis. The hypophysis divides and gives rise to two cells of which the apical cell takes part in the organisation of the dermatogen (Figs. 40 and 41). After this the lobing of cotyledons begins.

The mature seed contains the fully formed embryo surrounded by endosperm cells, which are completely filled with food materials and hence staining dark. A dark seed coat is seen to cover the contents of the seed.

7. Female gametophyte of *Nicotiana tabacum*

The morphological features are identical to those of *glutinosa* species, but for some details about the embryo-sac. A distinct tapetal layer was found lacking in the case of *tabacum*. The cells surrounding the embryo-sac are not differentiated from the rest and do not suggest the appearance of the tapetal layer (Fig. 43). Another peculiarity is in the nature of antipodals.

While the antipodals in the case of *glutinosa* are small and ephemeral (Fig. 29), those of *tabacum* are big with prominent nuclei. One of the antipodal cells is much longer than all others. The outline of these cells is seen long after fertilization, though the nuclei disappear. Antipodals of this nature have been described by Bhaduri (1935) in *Nicotiana plumbaginifolia*. But for these differences, the methods of development of female gametophyte and embryo are common to both the parent species, *tabacum* and *glutinosa*.

8. The development of megaspore in the *N. glutinosa* *N.* × *tabacum* F_1 hybrid

The hybrid has been reported to be sterile partly due to non-viable pollen and partly due to the abscission of flowers.

The development of the ovule is similar to that of the parents up to the commencement of meiosis in the megasporemother-cell.

The first division of the megasporemother-cell nucleus is very irregular. 28 bodies are seen, of which there are four bivalents centrally placed, all others being univalents, distributed all over the spindle region (Fig. 15). Such a behaviour has been described during the meiosis of the hybrid in pollenmother-cells, and has been discussed in detail (Raghavan and Srinivasan, A. R., 1941).

The dyad is formed (Fig. 16). Unlike the normal cases the two dyad cells do not undergo division simultaneously. Such a feature has been reported by Goodspeed (1923) in the embryo-sac mother-cells of *Nicotiana tabacum* × *N. sylvestris* F_1 hybrids. The chalazal cell divides first giving rise to two cells. Long after this, the micropylar dyad divides (Fig. 17). This stage was observed in ovaries from fully blossomed flowers, where, in normal cases, we ought to get the mature eight-nucleate embryo-sac stage.

The micropylar cell degenerates even before the end of the division, while the daughter cell of the chalazal dyad, at the micropylar end, shows signs of degeneration (Fig. 18). This latter cell degenerates soon and the megaspore is formed (Fig. 19). A peculiar feature however is the persistence of the nucellar layer up to this stage (Figs. 18 and 19). This suggests that neither the megasporemother-cell nor its daughter cells have increased in size to result in the degeneration of the nucellar layer.

Very soon after this even this megaspore degenerates as a dark mass in the embryo-sac region. The tapetal cells enlarge in size and the nucellus now disappears. All these stages are found in ovaries fixed from flowers one day after blossoming. After this, the ovule degenerates and causes the flower to be shed.

9. Summary

The development of microsporangium in *Nicotiana glutinosa* is described. Fusion of the tapetal nuclei has been recorded.

A comparative study of the embryo-sac and embryo formation in *N. glutinosa* and *N. tabacum*, the two parents of the present hybrid, has been made.

The development and behaviour of the megaspore in the hybrid is described and compared with that of the parents.

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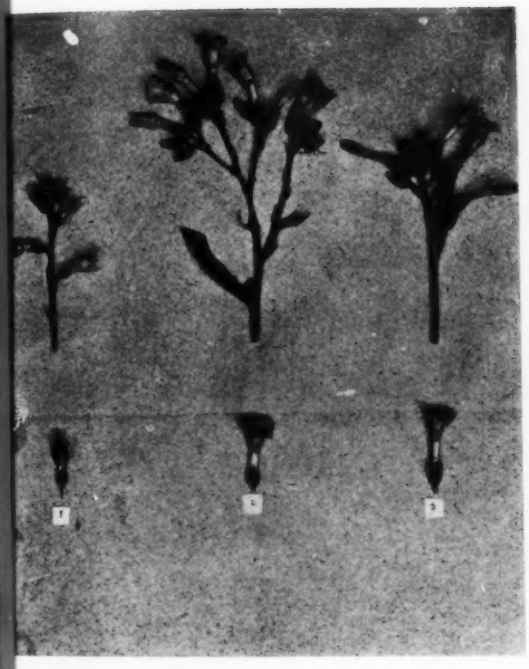

A

B

A. Shows the inflorescense and individual flowers respectively of (1) *N. glutinosa*, (2) F_1 hybrid *N. tabacum* × *glutinosa*, (3) *N. tabacum* var. *macrophylla* (Coimbatore).

B. Shows the general appearance of whole plant. (1) *N. glutinosa*, (2) F_1 hybrid *N. tabacum* × *glutinosa*, (3) *N. tabacum* var. *macrophylla* (Coimbatore).

SOME STUDIES ON THE POTENTIALITY OF SHRIVELLED WHEAT GRAINS

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Introduction

In the Punjab plains the growing period of wheat extends from November to April. Most of this time is occupied by the vegetative growth of the plant. The ripening of the grain is a rapid process and is completed within about a month on account of sudden rise of temperature commencing from about the middle of March. This is a very critical time for the development of wheat grain. Observations and records of past several years indicate that whenever there is hot and dry wind during the ripening period, the grain does not develop to its normal size. It gets desiccated and becomes shrivelled. Such imperfectly developed grains were found to have one-third of the weight of normal plump grains. It has also been observed that whenever there is a severe attack of rusts (*Puccinia glumarum* and *P. graminis*) or the crop has lodged, the grain is affected similarly. The yield of wheat in these conditions is reduced by nearly 20 to 30 per cent. In certain extreme cases a decline of 50 per cent. yield was observed.

The years 1928, 1932 and 1934 were very unfavourable in this respect and the crop suffered all over the Punjab Province. In several localities the entire produce consisted of shrivelled grains, and difficulty was experienced in obtaining good seed, for sowing purposes.

An investigation was taken in hand to study the potentiality of shrivelled grains with regard to their suitability for use as seed.

References

The results of experiments carried out on the influence of seed weight on yield have not been conclusive and workers on the subject hold divergent

views. Elder (1900) carried out experiments with wheat seeds weighing 50·9 and 33·9 grams per thousand grains respectively and found that heavy seeds produced heavier plants, having a larger number of productive stems and producing heavier and larger number of grains than plants from the lighter seed. Sanborne (1893), Latta (1891), Georgeson (1893) working on wheat have made it clear that from the practical point of view the balance of evidence is in favour of the use of larger seeds. Galloway (1894) who worked with radish believed that the large seed was superior to the small. Hicks and Dabney (1896) reached the same conclusion in experiments with Vetch, radish, rye and oats. Shamel (1904) working on tobacco says " Careful comparative tests of light and heavy seeds have proved that the best developed and the more vigorous plants are always produced by the big heavy seeds, while the small and light seed produces small, irregular and undesirable plants." Snyder (1904) in discussing light and heavy seeds of barley, wheat and oats, deduces from his observations that " the large seed is superior to small and the farmer is repaid by using heavy weight seed." Rotunno (1924), Hays (1893), Cummings (1914) hold that a distinct advantage follows the use of big and heavy seeds. Brenchley (1923) states that, " in the case of peas and barley, there is a steady rise in the dry weight of the plants as the initial weight of the seed increases." The interesting fact established was that the rate of percentage increase per day falls gradually as the weight of the seed rises, so that with prolonged periods of growth there is a tendency for the initial advantage gained by plants from the heavier seeds to disappear. Nielsen (1895) observed no marked difference in yield when equal weights of large and small grains of rye were sown.

Trelease and Trelease (1924) working on wheat in water culture state that " the yield of both tops and roots was found to be directly related to initial seed weight." Kiesselbach (1924) winding up several years work on seed size and yield of grains in wheat, etc., at the Nebraska station remarks "There will be no material and practical gain in the grain yield under farm conditions from the practice of grading small grain seed which is reasonably free from trash and inert matter."

Rudolfs (1923) while working on seeds *Phaseolus vulgaris* (Beans) states " Under these uniform conditions plants of seeds of a heavier weight show a decided advantage over plantlets from seeds of light initial weight." Voelcker (1900–04) working on wheat and barley found the yield to be slightly in favour of the small grains.

Codd and Martin (1933) working on rice in British Guiana, note that no significant differences in yield occur between the various grades of rice

seeds. They believe that most cereal seeds, and particularly rice have more than sufficient food to maintain the plant until it can assimilate for itself.

The Royal Agricultural Society Egypt in 1924, carried out a small experiment on wheat at Bahtim. It was found that there were no differences in yield due to the size of seeds.

Fikry (1936) while working on wheat in Egypt reports that there is a decided advantage in grading wheat. Luthra (1929) while working on shrivelled grain of wheat concluded " that shrivelled grains would prove good enough for sowing purposes for raising a fairly remunerative crop in years when unfavourable climatic conditions prevent normal development of wheat grains." He also observed that the progeny grains of plump and shrivelled grains were identical regarding consistency and size. Leggieri (1937) states " The experiment shewed that normal plants with normal plump grain were obtained from sowings of shrivelled wheat grains and the condition is not inherited." Johannsen's experiments (1908) on Princess beans show clearly that within a pure line large sized beans do not tend to produce large sized progeny and small sized ones small progeny, but all tend to reproduce the mean seed weight character of the pure line to which they belong. It is quite evident from the review of literature that the question of relation of size of seed to yield of grain is still undecided and more work is needed in this direction.

Experimental

The wheat used in all the experiments was the pure strain of " Punjab 9-D" (*T. vulgare*)—a selection, of the Botanical Section, Lyallpur, and introduced in the Punjab as improved wheat. The seed was obtained from a field near Lyallpur, of which the produce was wholly shrivelled. Normally developed plump grains of the same type were obtained from the Cerealist, Lyallpur, for use as control. The following investigations were made :—

1. Determination of the size, weight and germination capacity of the grains.
2. The vigour and metabolic acitivity of the seedlings raised from plump and shrivelled grains.
3. Subsequent development and growth rate of the progeny plants.
4. Yield.
5. Size and weight of the progeny grains.

I. Germination :

Germination percentage was determined on 100 grains of plump and shrivelled grains in ten lots in the laboratory. A similar study was made

under field conditions. The average results of the germination tests are given in Table I.

TABLE I

Showing percentage Germination of Plump and Shrivelled Grains of Punjab 9-D Wheat

Date of sowing	Kind of grain	Number of seeds sown	Germination							Total No. of grains germinated	Percentage germination	Standard (S.E.)
			19/11	20	21	22	23	24	25/11			
Laboratory studies (A)												
18–11–35	Plump	100	..	100	..	..	..	..	..	100	100·0	±0·00
	Shrivelled	100	..	53	20	11	4	3	2	93	93·0	±3·07
			$t = 2·2$ Insignificant									
Field studies (B)												
26–11–35	Plump	100								85	85·0	±5·71
	Shrivelled	100								78	78·0	±7·46
			$t = 1·0$ Insignificant									

Table I shows that the germination percentage of the shrivelled grains was $93·0 \pm 3·07$ as compared with 100·0 of plump grains. Though the difference is not significant, there is a striking contrast in the germination energy of the two kinds of seeds. The plump grains attained cent. per cent. germination in 2 days after sowing, while of the shrivelled grains 53% germinated in the same period. Moreover they took about seven days to reach the maximum germination against only two days required by plump grains.

Germination studies in the field were made in a well prepared soil and 500 seeds were sown at a depth of 3 inches in 5 lots. The germination was recorded 2 weeks after sowing and the results are given in Table I (B).

Percentage germinations were $85·0 \pm 5·71$ and $78·0 \pm 7·46$ for plump and shrivelled grains respectively. The difference is insignificant.

Absolute weight of grains.—Absolute weight of grains was determined by weighing 100 grains in 20 lots. The grains were taken at random. The results are presented in Table II.

TABLE II

Showing Absolute Weight of Plump and Shrivelled Grains of "Punjab 9-D" Wheat and "Punjab 8-A" Wheat

Year	Wheat	Kind of grain	Absolute weight of 100 grains in grams	S.E.	Difference Plump Shrivelled	Value of "t"	Remarks
1934	Punjab 9–D	Plump	3·912	±·199	2·634	9·02	Highly significant
	Do.	Shrivelled	1·278	±·058			
1935	Do.	Plump	3·944	±·106	2·716	24·03	Do.
	Do.	Shrivelled	1·228	±·041			
1938	Punjab 8–A	Plump	3·882	±·163	1·980	6·60	Do.
		Shrivelled	1·904	±·260			

The plump grains were 2·5 times heavier than the shrivelled grains. The difference is statistically highly significant.

Size.—The length and thickness of 100 grains was measured in milimeters in 10 lots by means of a Vernier Caliper. The average results are shown in Table III.

Table III indicates that shrivelled grains were much reduced in size as a result of adverse climatic condition. The average length and thickness of the plump grains were 6·25 × 2·89 mm. against 5·68 × 2·15 mm. of the shrivelled grains. The shrivelled grains suffered greater reduction in thickness, than in length.

TABLE III

Mean Size of Plump and Shrivelled Grains

	Plump grains	Shrivelled grains	Difference Plump Shrivelled	Value of "t"	Remarks
Mean length in mm. ..	6·25 ± 0·089	5·68 ± 0·158	0·57	3·35	Significant
Mean thickness in mm. ..	2·89 ± 0·047	2·15 ± 0·098	0·74	6·72	Do.

Density of grain.—The density of the grains was determined by means of specific gravity bottle using kerosine oil. The density is given in Table IV.

TABLE IV

Mean Density of Plump and Shrivelled Grains

	Plump grain	Shrivelled grain	Value of "t"	Remarks
Density ..	1·400 ± 0·04	1·342 ±0·02	1·45	Insignificant

The plump grains have higher density than shrivelled grains, but the difference is insignificant statistically.

II. The Vigour and Metabolic Activity of the Seedlings :

(*a*) *Seedling Vigour.*—In order to study the extent to which differences in size and weight of the parent grains are reflected in the subsequent growth and metabolic activity of the progeny seedlings, the following experiments were carried out.

The plump and shrivelled grains were sown in 100 sterilized dishes on blotting paper and were occasionally supplied with distilled water only. Increase in the dry weight of sprouts was taken as an index of seedling vigour. The dry weight determinations were begun when the sprouts (Plumule + radicle) were 2 days old and continued till the endosperm was completely depleted of the food material. Every time 300 seedlings of each group in 3 lots of 100 each were taken, severed from the unutilized endosperm and dried at 90° C. in an oven to a constant weight. The corresponding unutilized endosperm was also dried separately. The results showing the relative rates of sprout development and depletion of endosperm are set out in Table V and Fig. 1.

TABLE V

Days after germination	Mean dry weight of 100 sprouts in grams		Mean amount of unutilized endosperm		Remarks
	Plump grains	Shrivelled grains	Plump grains	Shrivelled grains	
2	0·179	0·055	2·950	1·143	Initial weight of grains was
4	0·572	0·382	2·532	0·903	100 plump grains= 3·374 grm.
6	0·963	0·474	1·903	0·616	100 shrivelled grains = 1·258 grm.
8	1·227	0·522	1·348	0·517	
10	1·339	0·559	1·217	0·413	
12	1·541	0·567	0·813	0·237	
14	1·619	0·574	0·606	0·208	

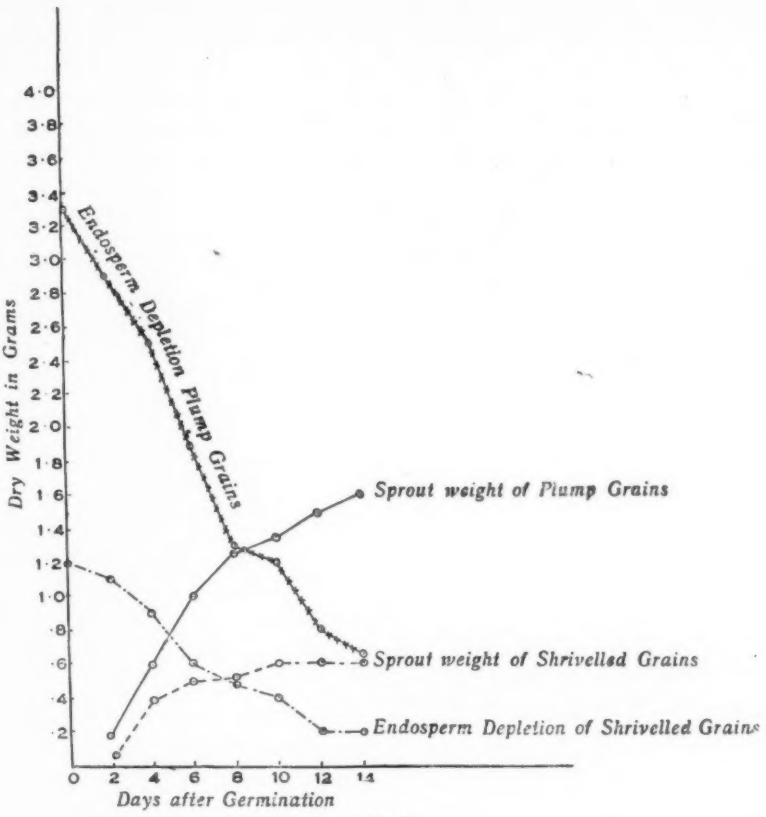

FIG. 1

Showing Increase in Dry Weight of the Sprouts of Plump and Shrivelled Grains during Germination and the Rate of Depletion of Endosperm

The results obtained (Table V) indicate that the average weight of seedlings of plump grains was greater throughout than the shrivelled grains. The plump grains therefore possess a higher potentiality for producing stronger seedlings.

(*b*) *Metabolic Activity.*—As respiratory activity of an organ is a direct measure of its growth and vigour, the rate of respiration of the seedlings was determined to compare their relative strength. 400 grains were taken and placed on the bottom of respiration jars (10″ × 5″) on a thick pad of cotton which had been previously sterilized and soaked with distilled water. The cotton pad served as reservoir of moisture for the germinating seedlings.

Distilled water was added after every 2 or 3 days. Fresh air was made to play on the seedlings which were in the dark by a continuous current method and CO_2 was absorbed in NaOH solution and measured by titration with N/10 HCl. Measurements of the respiratory activity were begun before the grains had chitted and continued upto 256 hours after germination. The results are given in Table VI and Fig. 2.

TABLE VI

Respiratory Activity of Plump and Shrivelled Grains during Germination
1*st set* (400 *grains*), 2*nd set* (400 *grains*)

Hours after Germination	Respiratory activity in terms of mgs. of CO_2 evolved per hour		Respiratory activity in terms of mgs. of CO_2 evolved per hour		Remarks
	Plump	Shrivelled	Plump	Shrivelled	
0	2·3	2·4	3·04	2·40	
24	5·16	2·5	7·09	4·45	Temperature of sets was
48	7·33	3·85	11·38	6·16	1st set 16°
72	11·00	6·05	15·76	7·70	2nd set 18°
96	11·21	8·41	13·01	7·70	
120	9·90	6·78	14·08	7·04	
144	13·00	9·60	19·61	10·00	
168	18·43	12·32	21·26	9·16	
192	21·60	10·00	18·50	7·7	
216	Nil	Nil	Nil	Nil	
240	17·60	7·90	17·60	7·48	
256	15·09	7·88	16·28	7·48	

The results obtained on two occasions independently show (Fig. 2) that except before chitting, the plump seedlings were metabolically more active than the shrivelled ones at every stage of germination and the shrivelled grains lagged behind considerably. The maximum respiratory activity in the plump seedlings was witnessed between 168–192 hours after chitting but the shrivelled seedlings touched the maximum a day earlier, which is evidently due to earlier exhaustion of their endosperm. Respiration results are in close agreement with the growth of sprouts. The growth rate of sprouts (Fig. 1)

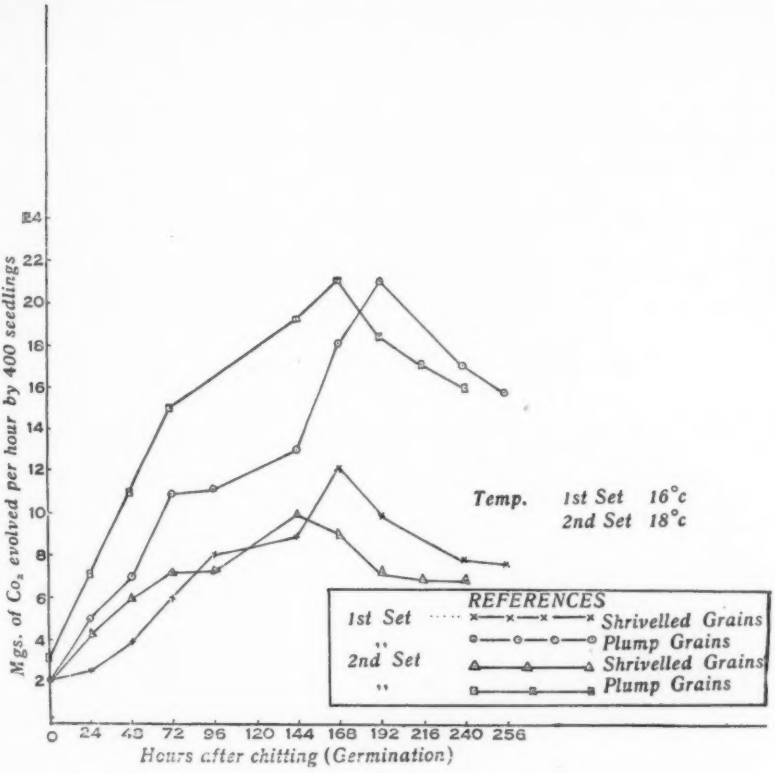

FIG. 2

Respiratory Activity of plump and Shrivelled Grains during Germination

also increase upto about 7–8 days after which the curves instead of rising run straight.

III. Development and Relative growth rate of the progeny plants under field conditions :

The data so far compared indicate that plump grains are more efficient in every respect. Blackman (1919) states that " a small difference in the efficiency indices of two plants may lead to a big difference in final weight." It remains to be seen how far this is true in the case of progeny of plump and shrivelled grains.

The effect of endosperm amount on the subsequent growth and development of the progeny plants was studied by taking plump and shrivelled grains

of " Punjab 9-D " wheat in the year 1934–35 and 1935–36 and growing them side by side in plots measuring 40 ft. × 10 ft. Seeds were dibbled by hand three inches deep at a distance of six inches between the plants and nine inches between rows. In order to secure uniform stand and avoid patches, which would otherwise have seriously influenced tillering and growth of the plants, two seeds were dibbled in each hole. When the germination had proceeded fairly well one seedling was pulled out, thus leaving only one plant in each hole. This resulted in a uniform stand of the crop.

Growth studies.—Since increase in height of plants does not afford an accurate measure of the growth of plants (Bailey and Trought 1927), it was decided to take increase in dry weight of the plants as a criterion of growth rate (Reed, 1924).

Dry weight determinations were begun when the plants were 10 days old in 1934–35 and 8 days old in 1935–36. The plants were dug out completely along with roots at regular intervals of 10 and 8 days respectively taken to the laboratory and washed free of soil with distilled water. The total dry weight of roots and tops was determined separately by drying them in an oven at 90° C. to a constant weight. The plants for dry weight determinations were taken at random. In earlier stages when the plants were young, about 100 of them were taken in 10 lots of 10 plants each but with the advance in age and subsequent increased size of plants it became difficult to manage bulky material and hence only one could be taken for each of the ten lots for the purpose. Afterwards the dry weight determinations were continued till the maturity of the crop. The results expressed as average dry weight per plant along with their S.E. are represented in Table VII.

Reference to Table VII discloses some interesting points. The seedlings raised from shrivelled grains, which had a lower initial weight on account of depleted endosperm put up very thrifty growth and showed lower dry weight per plant in earlier stages of development. The data show that the progeny plants of plump grains were significantly stronger and put up more vigorous growth. The marked difference in the size of plants between their progeny was noticeable upto the age of 60 days. Afterwards the progeny of shrivelled grains show a decided improvement in size and eventually make up the difference. They give evidence of their capability of producing a progeny as good as that of normal, plump grains in every respect. The dry weight of tops and roots equalized at this stage and no differences visual or otherwise were observed in the later stages. Plate V shows size of mature progeny plants of plump and shrivelled grains of Punjab 9-D wheat. Statistical examination of the data furnishes instructuve information.

TABLE VII

Days after germination	Mean dry weight per plant in grm. of the progeny of plump grains		Mean dry weight per plant in grm. of the progeny of shrivelled grains		Remarks
	Tops	Roots	Tops	Roots	
	Stem + Leaves		Stem + Leaves		
			1934–35		
10	·0169 ± ·0018	·0039 ± ·002	·0071 ± ·001	·0025 ± ·0015	
20	·039 ± ·0031	·0092 ± ·001	·0174 ± ·001	·0056 ± ·0021	Secondary roots begin to appear 1st irrigation given
30	·0695 ± ·0061	·0013 ± ·003	·0361 ± ·004	·0087 ± ·004	
40	·1120 ± ·0121	·0195 ± ·0055	·056 ± ·006	·0110 ± ·0018	
50	·275 ± ·032	·045 ± ·011	·1050 ± ·026	·023 ± ·0056	
60	·3516 ± ·146	·056 ± ·014	·3608 ± ·125	·055 ± ·023	
70	1·680 ± ·235	..	1·640 ± ·210	..	
80	2·794 ± ·935	·229 ± ·055	2·502 ± 1·170	·230 ± ·053	
90	10·49 ± 3·79	..	10·61 ± 3·59	..	Plants begin to ear.
100	18·29 ± 3·07	..	18·83 ± 2·42	..	
110	25·67 ± 3·82	..	24·44 ± 3·04	..	
120	25·249 ± 1·57	..	25·601 ± 2·00	..	
			1935–36		
8	·0169 ± ·0016	..	·0071 ± ·0011	..	
15	·0292 ± ·0018	·011 ± ·001	·0158 ± ·0016	·0045 ± ·002	
23	·0609 ± ·0037	·016 ± ·0012	·0249 ± ·0025	·0054 ± ·0018	
31	·0711 ± ·0022	·022 ± ·0021	·0356 ± ·0077	·013 ± ·0031	
39	·1817 ± ·0123	·031 ± ·0014	·0843 ± ·0110	·015 ± ·0020	
47	·343 ± ·038	·038 ± ·0018	·190 ± ·042	·031 ± ·0019	
56	1·480 ± ·11	·058 ± ·0031	·672 ± ·042	·057 ± ·0027	Dryweights of roots equalize
64	3·439 ± ·61	..	2·014 ± ·53	..	
72	7·032 ± 1·19	·226 ± ·018	7·89 ± 2·0	·230 ± ·020	Tops equalize
80	14·754 ± 2·2	..	15·056 ± 4·3	..	Plants show ears
88	21·820 ± 3·36	..	21·980 ± 2·8	..	
96	33·422 ± 3·96	..	32·322 ± 2·43	..	
104	40·8 ± 2·9	..	39·3 ± 2·9	..	

TABLE VII (A)

Showing Logarithms of Dry Weights of the Progeny of Plump and Shrivelled Grains in connection with Growth-rate Studies

Days after germination	1934–35		Days after germination	1935–36	
	Logarithms of dry weights of plants (Log 10ω) (y)			Logarithms of dry weights of (Log 10ω) (y). Plants	
	progeny of plump grains	progeny of shrivelled grains		progeny of plump grains	progeny of shrivelled grains
10	$\bar{2}\cdot2279$	$\bar{3}\cdot8513$	8	$\bar{2}\cdot2279$	$\bar{2}\cdot8513$
20	$\bar{2}\cdot5911$	$\bar{2}\cdot2405$	15	$\bar{2}\cdot4654$	$\bar{2}\cdot1987$
30	$\bar{2}\cdot8420$	$\bar{2}\cdot5065$	23	$\bar{2}\cdot7846$	$\bar{2}\cdot3962$
40	$\bar{1}\cdot0492$	$\bar{2}\cdot7482$	31	$\bar{2}\cdot8519$	$\bar{2}\cdot5514$
50	$\bar{1}\cdot4393$	$\bar{1}\cdot0212$	39	$\bar{1}\cdot2593$	$\bar{2}\cdot9258$
60	$\bar{1}\cdot5465$	$\bar{1}\cdot5575$	47	$\bar{1}\cdot5353$	$\bar{1}\cdot2788$
70	0·2253	0·2148	56	0·1461	1·8274
80	0·4462	0·3982	64	0·5364	0·3040
90	1·0207	1·0257	72	0·8471	0·8472
100	1·2622	1·2749	80	1·1685	1·1775
110	1·8894	1·3881	88	1·3389	1·3420
120	1·4116	1·4082	96	1·5240	1·5188
..	..	..	104	1·6107	1·6053

. The logarithms of the dry weights, when plotted against time give exponential, straight line curves, definitely following Blackman's (1919) compound interest law. Fig. 3 indicates data for relative growth rates of the progeny of plump and shrivelled grains for the year 1934–35 and Fig. 4 for the year 1935–36. The growth curves are inclined at a very small angle, showing thereby that there is not any marked difference between the relative growth rate of the progeny of plump and shrivelled grains, although progeny of shrivelled grains is having better of the two.

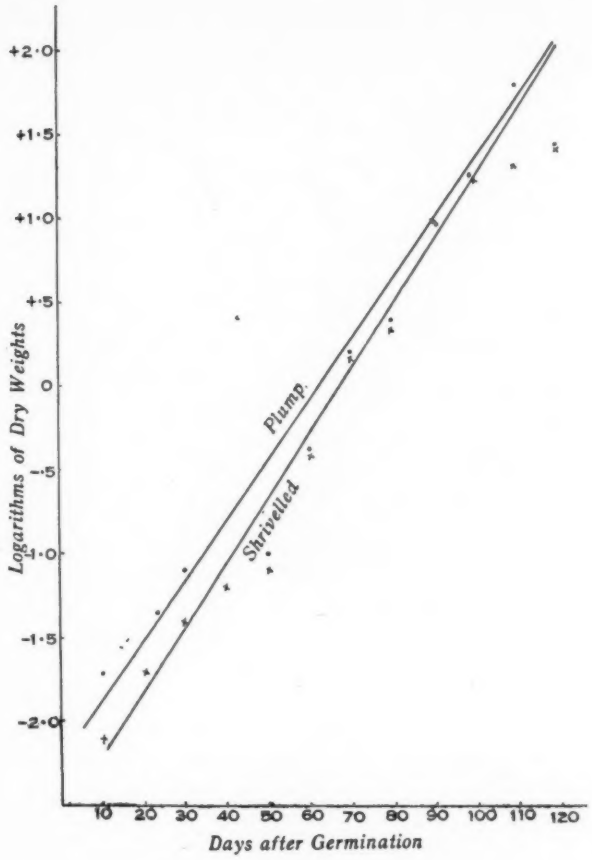

FIG. 3

Showing Exponential Straight line curves of the Logarithms of the Dry Weights when plotted against time

Exponential equations have been calculated by Fisher's method (1930) with a view to giving best fit to the observed dry weight curves. These equations take the form

$$W = A\, e^{bT}$$

where W = dry weight in gms.

A = a constant (the theoretical dry weight of seedling at time of germination).

e = the base of Naperian logarithms.

T = time interval in days from germination.

and b = the relative growth rate and efficiency index.

The calculated curves are shown in Fig. 3 and Fig. 4. The equations are shown in Table VIII along with the standard error of " *b* " in each case.

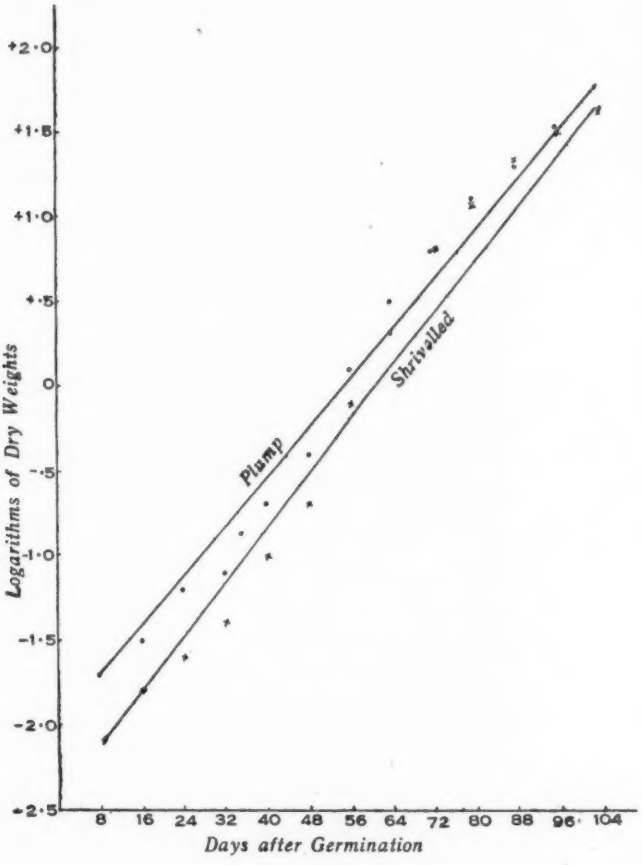

FIG. 4

Showing Exponential Straight line curves of the Logarithms of the Dry Weights when plotted against time

Reference to Table VIII indicates that shrivelled grains possess a slightly higher growth rate but the differences are statistically insignificant. A slightly higher growth accrued from shrivelled grains accounts for their ultimately making up all differences in size and growth, etc. The results of experiments also show that differences are not significant.

TABLE VIII

Showing Exponential Dry-weight Curves

Year	Type of plants	No. of observations	Equation $W = Ae^{bT}$	Relative growth rate = b	Standard error	Remarks
			7·616 T			
1934–35 ..	Plump	12	W=0·009314 e	7·616	0·0967	
			8·132 T			Insignificant
	Shrivelled	12	W=0·006858 e	8·132	0·0300	
			5·7608 T			
1935–36 ..	Plump	13	W=0·006822 e	5·7608	0·3131	
			6·1008 T			
	Shrivelled	13	W=0·007140 e	6·1008	0·6913	Insignificant

IV. Yield :

The yield of grain, which is the chief consideration of economic value in wheat cultivation mostly depends upon the amount of vegetative growth and other characters as marked below:—

1. Number of tillers per plant.
2. Height of plants.
3. Number of spikes per head or ear.
4. Size and weight of grains produced by the progeny plants.

The study of these characters which determine yield was made in 1934–35 and 1937–38.

1. Effective number of tillers per plant.—The tillering efficiency is determined by counting at several times during the life of a plant. The data were taken at maturity of the crop in 1934–35, and 1935–36 but in 1937–38, it was taken twice, once in January about two months after germination and again at maturity. 100 plants were always selected at random in plots, which were designed for comparing the yield of two types of grains.

The results of the tiller counts are given in Table IX.

Reference to Table IX indicates clearly that there is not much difference in the tillering capacities of the progeny of plump and shrivelled grains. In the year 1934–35 and 1935–36 the differences when statistically examined come to be absolutely insignificant. In 1938, however, the difference is in favour of the plump grain and is statistically only just significant.

TABLE IX

Type of wheat	Year	Time of counting tillers	Average number of tillers per plant of		Difference	Value of "t"	Remarks
			Plump	Shrivelled			
Punjab .. 9—D	1934–35	April "Maturity"	10·89	11·05	0·16 ±0·87	0·18	Insignificant
do.	1935–36	do.	12·67	13·38	0·71 ±0·876	0·81	do.
do.	1938	do.	14·41	11·76	2·8 ±0·94	2·8	Just significant

2. *Height of plants.*—Since height of plants is a measure of the vigour of the plant and is an important factor, which determines yield, it was thought fit to compare the height of the progeny plants as well. The height measurement data were taken in 1934–35. For this purpose 100 plants of each group were marked at random and the measurements were taken in lots of 10 each. The summarised results are given in Table X. In this connection it may be added that the height of the highest tiller was recorded for the sake of comparison.

TABLE X

Mean Height per Plant

Year	Average height per plant in feet		Difference	Value of "t"	Remarks
	Plump	Shrivelled			
1934–35	3·52	3·22	0·30 ±0·073	4·1	Just significant

The statistical examination of the results show that the progeny of plump grains is slightly leading the other group in height measurements and the difference is too small to be of any importance.

3. *Number of spikelets per head or ear.*—On the number of spikelets depends the length of ears, which subsequently determines yield. For comparing the number of spikelets in an ear, 100 ears were taken and these ears were of those tillers, which were marked out for height measurement records. The average number of spikelets per head are given in Table XI. The data when compared statistically show that the difference is highly insignificant.

TABLE XI

Year	Average number of spikelets per head		Difference	Value of "t"	Remarks
	Plump	Shrivelled			
1934–35 ..	20·188	20·084	0·104	0·093	Highly insignificant

4. Yield.—For comparing yield of the progeny of the two groups of grains, field scale trials were made in the year 1934–35 and 1937–38 in the case of Punjab 9-D wheat and Punjab 8-A respectively.

Data of 1934–35.—The size of plots was 40 ft. ×15 ft. *i.e.*, 1/400th of an acre. The seed rate was calculated on the basis of absolute weight of grains, and allowance was also made for the low germination capacity, of the shrivelled grains. The plots were systematically replicated thrice. In the yield trial plots the observations showed that the progeny of shrivelled grains lagged behind their rivals for about 60 days after germination. Afterwards the difference was made up and at maturity no discernible difference could be witnessed in the height and stand of the crop (Pl. V).

The plots were harvested and thrashed separately and the yield of grain obtained in seers is given in Table XII.

TABLE XII

Showing Average Yield of Plump and Shrivelled Grains

Year	Average yield in seers per Plot		Difference	Value of "t"	Remarks
	Plump grains	Shrivelled grains			
1934–35 ..	4·00 ± 0·91	4·03 ± 0·81	0·03	0·027	Insignificant
1937–38 ..	2·22	2·08	0·14 ± ·092	1·52	do.

The difference in yield of grains when examined statistically is insignificant.

In 1937–38 "Punjab 8–A" improved wheat was taken for comparison. There were 10 replications and the size of plots being 15 × 6 ft. In this case equal number of seeds, were dibbled by hand and in order to secure

uniform stand two seeds were dibbled in each hole and one being pulled out after germination and to eliminate gaps fresh seeds were also put in wherever needed. The crop from all the 10 plots was harvested and thrashed separately and the average yield of grain obtained is given in Table XII. The differences in yield are found to be highly insignificant.

5. *Absolute weight and size of the progeny grains.*—The data so far collected evidently show that shrivelled grains produce as good a progeny of normal plants of grain as obtained from plump grains in every respect. The weight and size of the progeny of parent shrivelled and plump grains were also compared. The data are given in Table XIII.

TABLE XIII

Absolute weight and size of the progeny grains of Punjab 9-D wheat 1934–35

	Average weight of 100 grains in grm.	Average size in mm.	
		Length	Thickness
1. Parent-shrivelled grains ..	1·228 ±0·041	5·68 ±0·158	2·15 ±0·098
2. Progeny grains of (1) above	3·835 ±0·29	6·08 ±0·085	2·67 ±0·03
3. Plump grains	3·764 ±0·31	6·18 ±0·13	2·80 ±0·02
Percentage increase in (2) above (1)	212·2	7·04	24·0

It is noteworthy that the progeny grains of both the parent groups are indentical as regards size and consistency. The weight of 100 progeny grains of the shrivelled parents is 3·835 ± 0·29 thus showing an increase of about 212·2%. There is also a corresponding remarkable improvement in size as well of the progeny grains of shrivelled parents. It is therefore concluded that the shrivelled grains give as good a progeny of plump, normal well developed grains, as can be obtained from plump, well filled grains. The results are exactly in conformity with the finding of Luthra (1929) and Leggieri (1937).

Discussion of Results

It has been proved beyond doubt that shrivelling leaves wheat grains much deformed and reduced in size (Table III) and weight (Table II). The reduction in the amount of endosperm however, does not adversely affect the viability of shrivelled grains. This has been substantiated by germination tests carried out in the laboratory and field. The germination energy has

been observed to be lowered greatly. The sprouts produced by the shrivelled grains showed retarded vegetative growth during early stages of development. This drawback was reflected in the fall of dry matter as compared with the sprouts raised from plump grains (Table VII).

The sprouts produced from shrivelled grains were also significantly metabolically less active and lagged behind in the output of CO_2 per hour (Table VI).

Under field conditions it has been noticed that the plants raised from plump grains have larger size and this advantage is maintained for about two months (Table VII). Greater growth, which the plants of plump grains make is evidently due to more "initial capital" in the form of endosperm which they possess. In later stages of development when the seedlings become independent of the food supply in the endosperm and on account of accelerated growth the differences in growth begin to disappear. The deficiency is gradually made up. At maturity both the crops present an equally intensive stand (Pl. V). The progeny grains are identical in form and weight. They are both plump and indistinguishable from one another. The growth-rate studies (Figs. 3 and 4) follow the compound interest law of Blackman (1919) and give straight line curves. The exponential equations when calculated give these a best fit to the observed dry-weight curves which strictly follow the equation, $W = A\, e^{bT}$.

The results have indicated that much of the growth of plants depends upon subsequent nutrition from soil, air and environmental factors, after the embryo gets a start in life by nourishment from the endosperm. Any initial advantage gained at the cost of endosperm does not last long. The whole of the endosperm is not in reality required by the young plant to attain the stage when it would be able to absorb nutrients from the soil and elaborate food substances for its growth (Luthra, 1929).

The yield trials have shown that shrivelled grains can give as good and remunerative a harvest as the plump ones and at maturity of the crop no visual difference can be detected in the height and stand of the plants (Pl. V). As stated before, the seed used for the experiments was of pure lines of types 9-D and 8-A. According to Johannsen (1908) the average yield of plants raised from large and small grains of pure strains is about the same. The results reported fall in with the data and hypothesis of Johannsen's work on Princess beans. These results corroborate the data collected in 1934–35, 1935–36. The shrivelled grains produce a normal crop of well developed grain (Table XIII) and the results are exactly in accord with the findings of Luthra (1929) and Leggieri (1937). Luthra (1929) also found that by sowing

half cut grains a crop of normal growth and normal plump grains was produced. It may therefore be inferred that the shrivelled grains have the same genetic constitution and potential power as the normal, plump grains. The difference is only in the make and size, which is greatly the result of fluctuating environmental factors and it does not affect hereditary potential power.

Consequently there is no advantage in grading wheat for selecting large sized grains for sowing purposes with a view to getting increased yield.

Summary

1. The prevalence of hot and dry winds during the development retard the growth in size of the grain and cause its desiccation with the result that it gets shrivelled and there is a decline in yield. The grain is much reduced in size and is deficient in food store.

2. It has been substantiated by experiments that germination power and suitability of shrivelled grains for sowing are not affected.

3. The progeny plants of shrivelled grains lag behind the plump grains in growth in early stages of development, but the initial differences disappear at a later period. Ultimately shrivelled grains produce a crop as good as obtained from plump and normal grains.

4. The seedlings produced by shrivelled grains are metabolically less active than plump grain and lag behind considerably throughout the germination period.

5. The initial lower weight and smaller size of the shrivelled grains do not operate adversely on the subsequent process of growth and yield of the crop.

6. The shrivelled grains produce a normal crop of well developed grains and the progeny grains show an increase of 212·2 per cent. in weight over the parent grains.

7. It is shown that there is no advantage in grading wheat in order to get increased yield in the produce of pure line.

Progeny of Shrivelled and Plump Grains of Punjab "8 A" Wheat, 1937-38

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FIRST RECORD OF AN AMŒBA PARASITE OF AN INDIAN TERMITE

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Received December 23, 1940

Among the protozoa which constitute the most interesting fauna inhabiting the intestine of some genera of Termites, amœbæ, however having been seen by Cleveland in 1923[1] have been definitely described and figured by Kirby.[1] There are actually the following species recorded :

Hosts	
Mirotermes hispaniolæ Banks	*Endamœba disparata* Kirby (1927) *Endamœba majestas* Kirby (1927) *Endolimax termitis* Kirby (1927)
Mirotermes panamænsis Snyder	*Endamœba simulans* Kirby (1927) *Endamœba sabulosa* Kirby (1927)

In a further paper Kirby[2] added to the above list :

Type Host	
Amitermes Beaumonti Banks	*Endamœba beaumonti* Kirby (1932)

Additional hosts : *Amitermes coachellæ* Light, *A. minimus* Light, *A. Wheeleri* Light, *A. medius* Banks.

Kirby says[1] that minute amœbæ, in such a small number that it was necessary to search for them carefully, were found in *Amitermes beaumonti* Banks (described later on as *Endamœba beaumonti*), *Amitermes medius* Banks, *Subulitermes Kirbyi* Snyder and *Cornitermes acignathus* Silvestri.

All these have been recorded in America (Călifornia, Barro Colorado Island and Panama) and have the following characters, according to the resumé given by the author himself.

Genus Endamœba :

E. disparata.—20–40 Microns diameter, average 32. Broad, blunt pseudopodia. Cytoplasm crowded with particles of wood and other material. Nucleus about 8 microns in diameter, with distinct membrane, several peripheral nucleoli and an irregular mass of chromatin occupying a large

part of the inner region. Mitosis with 12 varicose filiform chromosomes formed from a spirena. Apparently no centrosome or spindle fibres and no definite equatorial plate. Xylophagous.

E. majestas.—65–165 Microns in longest diameter, aver. 115. Short lobose pseudopodia often protruded from various parts of the body. Endoplasm crowded with food material, mostly fragments of wood. Nucleus 15–26, aver. 20, with irregular reticulum on which are many fine granules ; a dense aggregation of chromatin granules near centre; a deeply staining granule in a clear area within this aggregation, and irregular, often anguilliform, vacuolated masses of nucleolar material in outer zone. In division the nucleoli disappear and the chromatin granules aggregate into threads. Xylophagous.

E. simulans.—50–150 in longest diameter, aver. 77. Many short, blunt pseudopodia, protruded from various parts of the body. Endoplasm contains ingested wood but less than *E. majestas*. Nucleus 15–20 aver. 17,6 with two intranuclear zones, the outer clear, the inner dark. Chromatin in an irregular, karyosome-like mass in inner zone ; small peripheral nucleoli at the periphery of this zone. In mitosis inner zone expands, nucleoli disappear, chromatin granules form threads which become dispersed within the nucleus. Xylophagous.

E. sabulosa.—19–35 in longest diameter, aver. 26. Ingested food material not abundant. Endoplasm contain a large number of granules which stain deeply with iron hæmatoxylin and resemble some mitochondria in size and form ; they may be specially abundant about the nucleus. Nucleus 7–10, irregular mass of granular chromatin occupies most of the central zone, several rounded nucleoli at periphery of this. In some individuals chromatin is more concentrated in nuclei which may be single or, less commonly, in couples closely applied to one another. Xylophagous.

E. beaumonti. Length 11–37, width 10–21, lobe-like pseudopodia formed by expulsive, intermittent movements ; nucleus 4,5–7 with large granular central chromatin mass with karyosome ; scattered chromatin granules or a few large granules beneath nuclear membrane. Feeds on bacteria.

Genus Endolimax :

E. termitis. Shape, when regular at all spherical, ovoidal or ellipsoidal; usually no definite speudopodia ; 9,5–27, av. 16. Cytoplasm dark, granular, ingested particles of wood few. Nucleus 4–5 with large central karyosome and many chromatic granules, under the nuclear membrane. Xylophagous.

Indian termite harbouring the present amœba.—The present amœba has been studied only in slides fixed with Schaudinn's acetic sublimate and Bouin (wet fixation) and stained with Heidenhain's iron hæmatoxylin. The preparations were made for demonstration to my pupils of the Protozoan structure and the termites were not kept for identification, as I am aware of the types of Protozoa found in the termites of this region of India. The abundant Devescovinid fauna with its three species and the Metastesphanonympha which are in the intestinal contents of this termite led me to believe that the termite was the species *Coptotermes heimi* which harbours in Gôa such kind of protozoa. From my former studies I have arrived at the conclusion that every termite possesses its own fauna and it is possible to identify termites by their parasites. This idea I have definitely stated in one of the conclusions of a paper presented to the Twelfth International Congress of Zoology : " nous

nous croyons authorisé á proclamer que chaque termite a sa faune particulière qui lui est entièrement propre et que d'après la spécifité de leur faune on pourra identifier les termites en question ".[3] The same idea has been expressed by Kirby when he says[1] speaking of the amœbæ of *Mirotermes hispaniolæ* and *M. panamænsis* : " they (the amœbæ) differ sufficiently in the two hosts to be readily distinguished, at least when stained much more readily than their hosts. Indeed they may serve as a means of separating the species of insects which morphologically resemble each other closely."

I am quite sure that in a detailed study of the parasites of *Coptotermes heimi* Wasm I never found any amœba at all, and having not kept the actual termites for identification on account of the Devescovinid and Calonymphid fauna, believing it to be the same *Coptotermes heimi*, I am rather inclined now to suppose that the termite habouring this amœba, besides the Devescovinid and Calonymphid flagellates, must be some *Coptotermes* closely allied to *C. heimi*. This is the reason why in the epigraph of this paper I say " an Indian termite ", waiting for an opportunity of finding again such colony to have the host duly identified by some specialist.

Morphology of the amœba in stained specimens.—As we have said, the study of the amœba has been done only in stained specimens. Its shape is oval or ellipsoidal, in some cases almost regularly circular, very seldom somewhat irregular. The first thing which strikes in this amœba is its membrane which is definitely periplastic and possesses a thickness which I have not seen in any other amœbæ. A delicate but distinct periplast has been described by Kirby in *Endamœba majestas* and also in *Endolimax termitis*, although the author believes referring to this last species, that in many cases " this appears to be a result of the preservative treatment ".

On account of such a strong constitution of the membrane, indeed, there are usually no marked pseudopodial protuberances. The pseudopodia are seen rather as narrow and irregular lobose expansions of the protoplasm, noticeable only on account of their less siderophyl outline (Figs. 1, 2, 4). In most cases, the uniformly dark stained cytoplasm fills completely the inner area of the body showing sometimes in the periphery a more or less vacuolated appearance (Fig. 3), with the vacuoles free of inclusions.

The structure of the cytoplasm is, naturally, vacuolated, but it is not possible to see the net of this vacuolisation, as in many other amœbæ. Generally, whatever be the degree of the differentiation of the iron hæmatoxylin, what we see is a cytoplasm rather uniformly stained and stuffed with inclusions.

These inclusions are mostly roundish bodies, more or less darkly stained, some of these with a membrane around, others with a net of nuclear structure,

showing that such bodies are probably remnants of animals phagocited by the amœba.

There are also minute bacteria and chromatic granules resembling as in *Endamœba sabulosa* the mitochondria of *E. gengivalis*. In one specimen we found a crystal (Fig. 4) and in many of them parasites of the genus *Sphærita* (Figs. 3, 6). No particles of wood were however seen in the cytoplasm of this amœba.

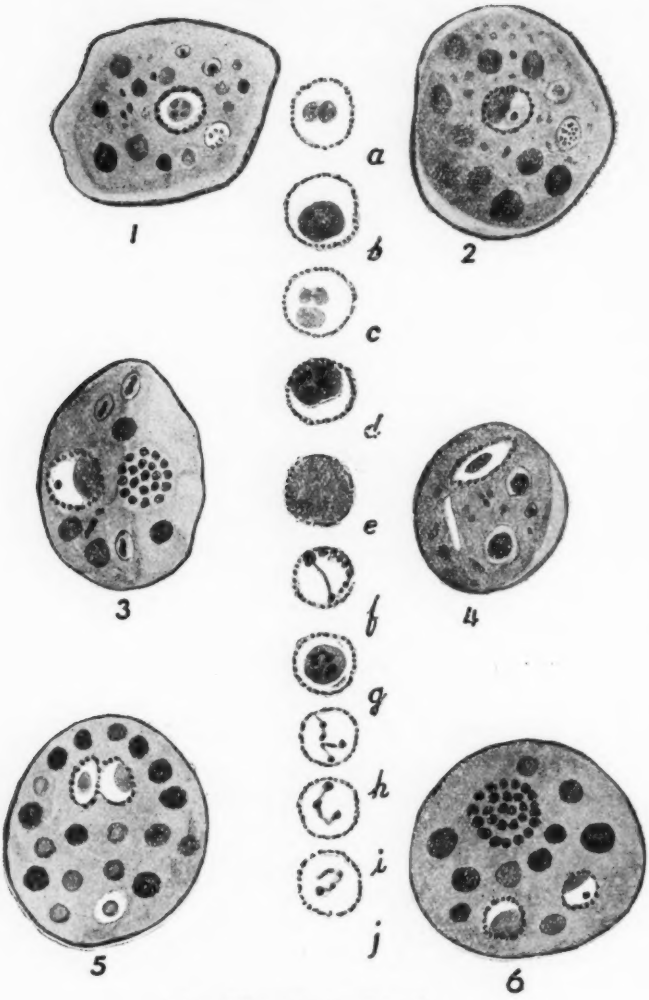
The nucleus of this amœba has a very peculiar structure. Circular and occupying various positions in the body, sometimes at the periphery, sometimes in the centre, its membrane is constituted by a row of dark stained chromatic granules. Such a deeply staining granular ring forming the boundary of the nucleus has been described by Kirby in *Endolimax termitis*. This appearance is uniformly seen in every specimen and when the amœba is filled up with round inclusions, covering the whole endoplasm, one can discover the nucleus, at once, by the above described appearance.

Inside the nucleus there is an irregularly outlined Karyosome, faintly stained and apparently composed of plastin, in which are imbedded two to four granular masses, a little darker, but never possessing the siderphylia of the chromatic ring (Fig. 1 and *a*, *b*, *c*, *d*). This Karyosome may fill completely the inner area of the nucleus (Fig. *e*). No reticular area as figured by Kirby in *Endolimax termitis* can be seen here even in the best stained individuals.

In more than 500 amœbæ studied, only once, the nucleus was spindle shaped, with nothing, however, which might suggest some divisional stage. Fusiform nuclei in a very large number of amœbæ have been described by Kirby in *Endamœbæ disparata*.

In a number of cases a strongly siderophyl granule, totally independent of the Karyosome, and surrounded or not by a vacuole has been seen in the interior of the nucleus (Figs. 2, 3, 6). I can hardly suggest an interpretation of such structure which is not a constant feature of these nuclei. It is not indeed homologous to the centriole of the nuclei of *Entamœba*. It is not also similar to the large granule surrounded by a clear area which Kirby has described at the centre of the chromatin mass of *Endamœba majestas* and suggested to be possibly homologous to the small Karyosomes of *Entamœba* and *Dientamœba*. It seems to be of the same kind as the " minute central granule set apart by a surrounding clear space " which Kirby described in *Endolimax termitis* and figured in his sketches 31 and 32, Pl. 23.

We have not been able to see any mitotic figure, the only definite divisional forms being binucleate amœbæ (Figs. 5, 6) with the daughter nuclei very



1-6 = different types of Ameba.
3 and 6 with Sphaerita
a-j = nucleus of Ameba.
Endolimax goheeni n.sp.

close to or already separated from one another. The nuclear structures figured by us in *f-j* does not give evidence of miotic stages. Our Fig. *f* corresponds perhaps to the intradesmose described by Kirby (his Fig. 33, Pl. 23) in *Endolimax termitis*. If the structures figured by us in *g-j* are to be

interpreted as early prophase stages, the number of the chromosomes should be only four. But we are very doubtful on this point and we prefer for the moment to keep a prudent reserve and to state that the type of mitosis in this amœba remains unknown.

Measurements.—Circular forms circa 10–12 microns diameter; elliptic forms length generally 18—width 15; nucleus 4, 5 microns diameter.

Classification.—The nuclear structure of our amœba is similar to the amœba of the genus *Endolimax*, the only difference being that the Karyosome does not stain so strongly as in other representatives of this group.

It is very similar to *Endolimax termitis* Kirby from which it differs: (1) by its measurements; (2) by the inner structure of the nucleus which does not show any fibrillar net connecting the Karyosome to the nuclear membrane and by the number of Karyosomic masses which do never exceed four; (3) by the thickness of the pellicle,(4) by lack of wood particles in the endoplasm.

We consider, therefore, this species a new one which we will devote to Dr. R. Goheen, Surgeon to American Mission Hospitals, first in Vengurla and now in Miraj, naming it *Endolimax goheeni* n.sp.

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REVISION OF THE FAMILY *DEVESCOVINIDAE*, ITS GENERA AND SPECIES, WITH RECORD OF THE INDIAN SPECIES OF *DEVESCOVINA*

By Prof. Col. I. Froilano de Mello
(Nova-Goa)

Received December 2, 1940

The enormous material to be found in India where the termites are so abundant leads me to make a revision of the protozoan fauna harboured in the intestine of some of these species hoping that such a revision will be of some help to the protozoologists working in this country and that their attention will be called to such a wide and still scarcely explored field of scientific research.

For rendering this paper more instructive, it will be illustrated with semi-diagrammatic figures of the different genera and species included in a family and the writer will be grateful to any scientist who may help him in this task, lending him any paper on termites parasites, which he may possess, in order to make this research the most complete possible.

Family Devescovinidæ *Poche* 1913 (1)

Diagnosis.—Usually three anterior flagella. One trailing flagellum which is stouter and usually longer than others, free or partially adherent to the body. Cresta near the base of the trailing flagellum, under the form of a small subtriangular or rod-like organel, which may become a large internal plate in some genera. Axostyle well developed. Parabasal body, in most species, coiled from one to many times around the axostyle behind the nucleus. Hosts : mostly intestine of termites.

Genera :

(A) *Devescovina* Fôa 1905. Three (sometimes two) anterior flagella. One long and thick trailing flagellum with a cresta attached to its base ; axostyle with a parabasal coiled around behind the nucleus.

Sp. typ. *D. striata* Fôa par. of *Coptotermes grassii* (Fig. 1).

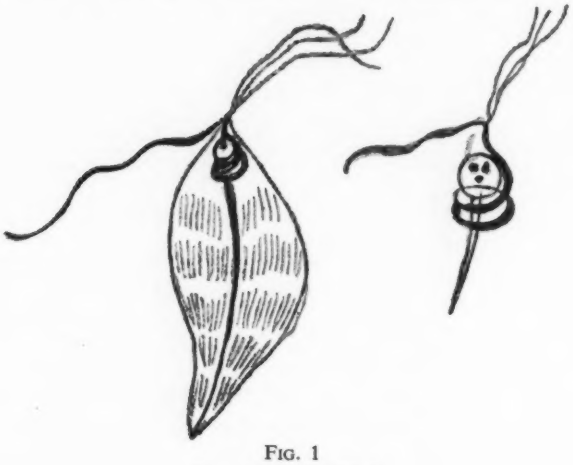

FIG. 1

D. striata (after Fôa)

(1) Fôa[2] studied the material sent from Iquique (Chile) by Prof. De Vescovi. Her description of *D. striata* is here resumed: Pyriform, average measurements 40 microns × 20. Ectosarc finely striated in longitudinal sense, the striæ being interrupted and not continuous through the whole length of the body. Axostyle beginning at a slight distance from the ant. pole and running till the posterior one, which it may exceed, protruding outside the body. Parabasal starting from the ant. pole, running close to the nucleus and making behind it a double collar surrounding the axostyle. Three ant. flagella. One trailing flagellum, band-like. (Fôa did not describe neither figure the cresta).

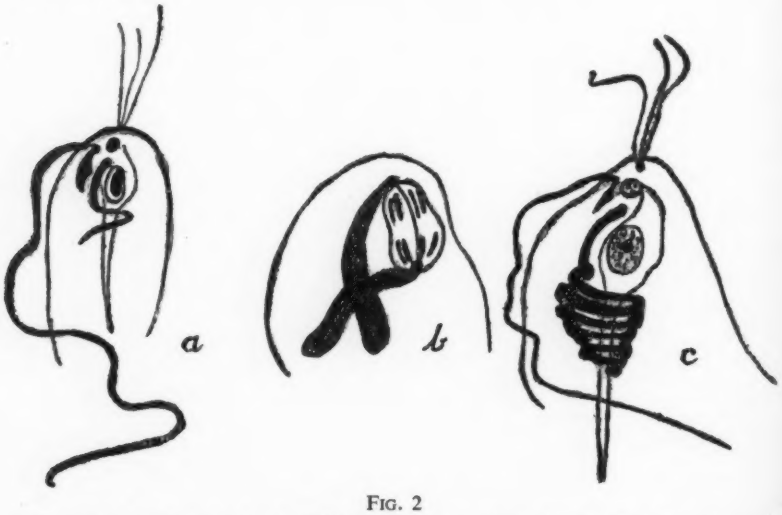

FIG. 2

a, *D. striata* var. *hawaiensis* ; *b*, division of nucleus and parabasal. Paradesmose very distinctly seen ; *c*, another specimen showing a large number of coils of the parabasal.

Other Species

(2) Janicki[3] figures the cresta of *D. striata* Fôa, which is believed by Kirby to be homologous with the basal fibril of the undulant membrane of Trichomonads and gives a full description of *D. striata* var. *hawaiensis*, par. of *Calotermes castaneus* from Honolulu (an identification which is wrong according to Kirby, the termite being *Neotermes connexus*). 45–55 microns × 10–25. Discontinuous longit. striation on the ectosarc. Axostyle in whose ant. portion the nucleus seems to be imbedded. Parabasal with 2-3 turns around the axostyle in slender forms, 3–8 in larger ones. Three ant. flagella starting from one blepharoplast. Trailing flagellum long, flattened, with a cuticular sheet at its base, named by the author *Schlephgeisselscheide*. Mitotic nuclear division with a distinct paradesmose.

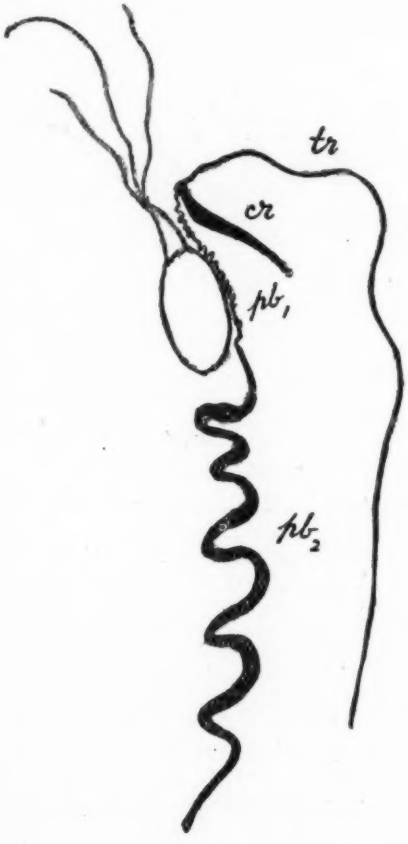

FIG. 3

D. pruvoti: *cr*, cresta; *tr*, trailing flagellum; pb_1, first spiral portion of parabasal; pb_2, second coiled portion of parabasal (after Duboscq and Grassé).

(3) Duboscq and Grassé describe their *D. pruvoti*, par. of a *Calotermes* from Lifou (Loyalty Islands). Leaving aside their ideas on the so-called *Trimitus stage* in the evolution of *Devescovina*

and other flagellates, ideas with which we do not agree, *D. pruvoti* is characterised by the special form of the parabasal, with its first portion, an eosinophyl thread, finely spiralated and with included syderophyl grains closely attached to the nucleus, and the second portion with a variable number of coils. The so-called large forms measure 75 microns × 36 and possess two blepharoplasts, one for the ant. flagella and the suspensor lamina, the second for the trailing flagellum, cresta and parabasal (Fig. 3).

FIG. 4

D. polyspira (after Lewis)

a, b, vesicles and splitting of the parabasal ; *c,* division with paradesmose

(4) *D. polyspira* Lewis 1933, par. of *Kalotermes occidentis,* from Lower California and Mexico. Ovoid or pyriform, averaging 21–45 microns. Oval nucleus with an eccentric karyosome and often a cone of darkly staining material projecting from the chromatin material towards the axostyle. Three ant. flagella. Trailing flagellum slender, round and tapering at both ends. All flagella 1½ times the body length. Sub-triangular cresta 1½–3 times the diameter of the nucleus, often projecting in a tapering point encompassed by a syderophyle muff at the point of the emergence from the body. Parabasal with ¾ to 2¼ æotropic turns about the nucleus and axostyle, *distally often split into as many as* 6 *to* 8 *strands,* connected to the blepharoplast by a peripheral parabasal thread which extends throughout its entire length. Clear area surrounding the nuclear region (Fig. 4).

Kirby believes that *D. polyspira* should be removed to the genus *Metadevescovina* and he names it *M. polyspira.*

FIG. 5

D. hilli (after Duboscq and Grassé)

(5) *D. hilli* Duboscq and Grassé 1926, par. of *Glyptotermes iridipennis* from Australia.[6] 35–45 microns long but small forms of less than 20 microns. Ectosarc without striation, but a pseudo-striation may be noticed, due to the fixation of spirochætes on the pellicle. These spirochætes are particularly fixed in a great abundance near the rostrum of the large forms between 35–45. On still larger forms (? 40–55) the spirochætes disappear and are substituted by fusiforms[7]; syderophyl grains on the pre-nuclear region. Parabasal with a tight coil near the superior nuclear pole and 2½ turns on the inferior pole, the last one being looser and ascending again on the middle nuclear zone (Fig. 5).

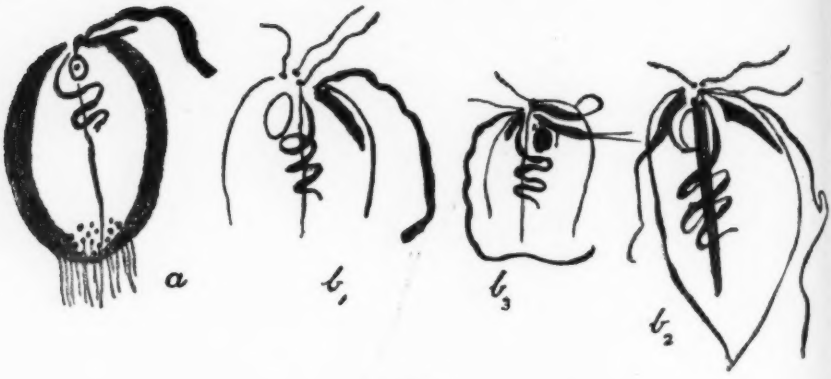

FIG. 6

a, *D. stereociliata*; b_1, *D. glabra* ; b_3, with two papillæ; b_2, with one papilla (after Grassi)

(6) *D. stereociliata* Grassi 1917,[8] par. of *Glyptotermes parvulus* from Goald Coast. Oval, ectosarc with not very distinct transverse striation and on the posterior pole a tuft of immobile *stereocilia*, which Kirby believes to be bacteria attached,[9] an opinion to which I cannot subscribe, from what I have seen in some *Holomastigoides* from *Leucotermes*. Axostyles, parabasal with two turns in the figures but in the description it is written : describing a half circle around the nucleus, extends up to the origin of the axostyle. Trailing flagellum thick, membraniform, with a closely attached cresta. One anterior very delicate flagellum described and figured. On the ant. extremity a group of syderophyl granules (Fig. 6 *a*).

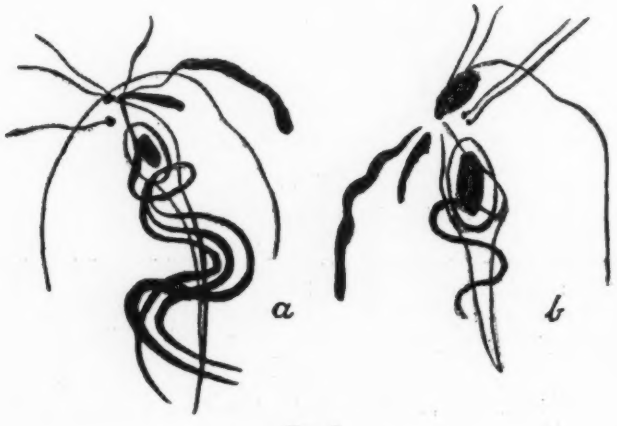

FIG. 7

D. nova: *b*, papilla shown (after Grassi)

(7) *D. glabra* Grassi 1917, par. of *Cryptotermes havilandi*, from Nigeria.[8] Resembles the former species, but possesses a papilla from which start at least two flagella. Sometimes, it seems that the papilla is double. Membraniform trailing flagellum, subtriangular cresta, which seems never to project from the body. Axostyle. Parabasal with 2-3 turns, starting from a point very close to that from which emerge the cresta and the trailing flagellum. Dimensions according to Kirby 57 × 22 microns (Fig. 6 *b*).

FIG. 8

D. lemniscata (after Kirby)

FIG. 8 (*a*)

D. elongata (after Bernstein) schima

(8) *D. nova* Grassi 1917, par. of *Neotermes erythraeus* from Erythræa.[8] Axostyle starting from the anterior extremity and gently curved around the nucleus, whose posterior pole it surrounds. Papilla with four delicate anterior flagella. Training flagellum stout but not flattened. Parabasal long, large, extending some distance into the body and *distally separated into several filaments*. Crest. Dimensions after Kirby[9]. 58 × 27 (Fig. 7).

Kirby recently[17] has discarded it from the genus *Devescovina* and included in the genus *Caduceia* as *C. nova*.

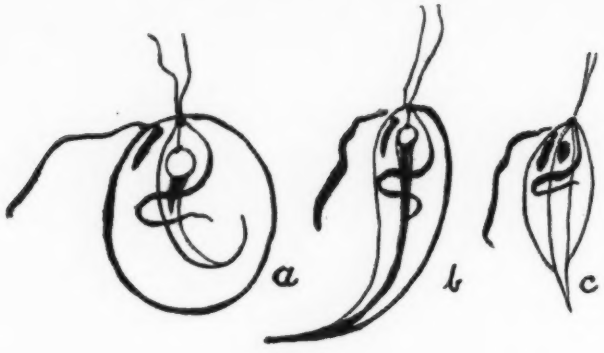

FIG. 9

Devescovina of *Coptotermes heimi*

a, *D. damanensis* ; *b*, *D. cometoides* ; *c*, *D. Kirbyi* (after de Mello and Brito)

(9) *D. lemniscata* Kirby 1926,[9] par. of *Cryptotermes hermsi* from Fanning Islands, Central Pacific Ocean. Average size 28 × 11 microns. Oval or piryform, frequently with projecting axostyle. Rostrum with three delicate flagella. Training flagellum band-like, broad. Parabasal narrowed proximally, broad at distal end, with 1–2 turns and a parabasal thread running through it. Crest. Discontinuous striation (Fig. 8).

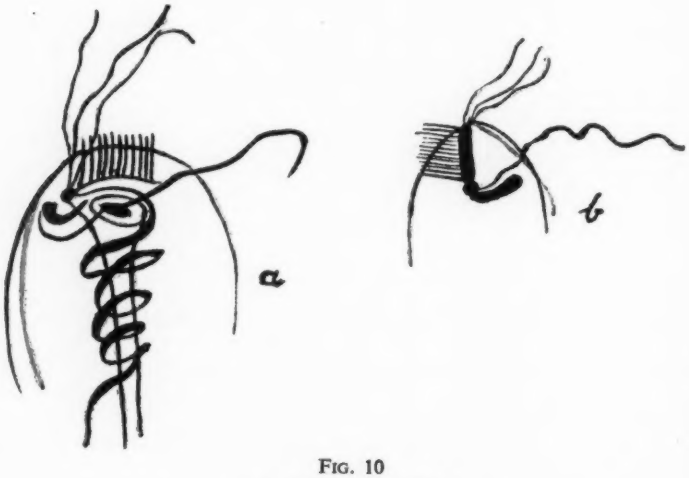

FIG. 10

Metadevescovina debilis

a, front view ; *b*, side view ; showing the tuft of short flagella (after Light)

(10) *D. elongata* Bernstein, par. of *Hodotermes murgabicus* from Turkestan (Fig. 8 *a*). This flagellate which Duboscq and Grassé considered a species of *Polymastix* has been related by Kirby[11] to the genus *Devescovina*, after studying a very similar parasite in *Anacanthotermes* (*o*) *characeus*.

Almost cylindric 30–77 × 3–6 microns. Longitudinal or oblique striation. Very slender axostyle. Parabasal with one turn. No trailing flagellum but four anterior flagella, three of which starting from one basal granule and the fourth from another. Kirby remarks that in the parasite of *Anacanthotermes* there is a cresta, probably not noticed by Bernstein and that one of the flagella is a trailing one.

Indian Species of Devescovina par. *of Coptotermes heimi* (according to the identification of the termite made by Dr. Baini Prashad. Kirby believes that there is a mistake[12] as from what is known from termites Protozoa the host must belong to *Calotermes*).

D. damanensis
D. cometoides } de Mello and Brito 1929.
D. Kirbyi (13, 14)

Kirby, who has examined my preparations, informs me, in a private letter, that he believes the last species to be rather a *Janickiella*. (I do not agree with his opinion.) The general characters of these three Indian *Devescovina* are : Ectosarc with discontinuous longitudinal striation. Nucleus anterior, with centrosome attached to the nuclear membrane or embedded in the endosome and united through a rhizoplast to the basal granule from which start the axostyle, the parabasal and the anterior flagella. Cresta falciform and a large and long membraniform trailing flagellum, both emerging from the second basal granule. Axostyle with axoneme, embraces the posterior nuclear pole and has often in its interior a syderophyl triangular area attached to the nuclear membrane. Parabasal with one or many turns around the axostyle. Two anterior flagella, one being longer than the others.

Specific characters (Fig. 9) :

(11) *D. damanensis*. Roundish, with a recurved non-protruding axostyle. Length min. 9, max. 70 ; 77% between 24–38 microns. Breadth min. 6, max. 45. Trailing flagellum min. 40, max. 120, average between 80–60. Cresta 6–8. Ant. flagella circa 25.

(12) *D. cometoides*. Pisciform, ending generally in a long tail. Axostyle with a very syderophyl axoneme running till the end of the body. Length min. 30, max. 100; 77% between 40–64 microns. Breadth min, 60, max. 26. Trailing flagellum, min. 45, max. 80, average between 60–70. Cresta 6–8. Anterior flagella circa 25–30.

(13) *D. Kirbyi*. Pyriform, with a gross tubular, eutrichomastoid axostyle, longer than the body of the parasite and protruding through its posterior pole. Length (total, comprising the posterior tip of the axostyle) min. 12, max. 88; 63 % between 15–25. Length of the body alone, without the axostyle's posterior tip, min. 7, max. 55; 69% between 10–16. Breadth min. 3, max. 16. Trailing flagellum min. 30, max. 90, average between 40–60. Anterior flagella circa 20–25.

(B) *Metadevescovina* Light 1926.[15] Idem as *Devescovina*. Possesses moreover on the anterior pole a fibril in connection with the anterior flagella and from which emerges a tuft of short flagella, very distinct by their siderophylism from the spiral organisms attached to the body of this flagellate.

Sp. typ. *M. debilis* Light 1926, par. of *Kalotermes hubbardi* (Fig. 10) from Banks of Arizona and California.

M. debilis. Diagnosis apud Light. Moderately large, xylophagous polymastigote similar in most characters to *Devescovina*. Three approximated secondary (anterior) flagella and a primary (posterior) flagellum in the nature of a trailer. An intracytoplasmic fibril associated with the bases of the secondary gives rise to a number of short tertiary flagella. A short curved chromatic basal rod underlies the base of the primary. Nucleus anterior, transverse, connecting by a neck

with the neuromotor centre. The axostyle extends throughout the body, ending anteriorly in a loop which integrates with the neuromotor system. The parabasal body is large, long, in a loose left spiral about the nucleus and axostyle. It contains a central thread which integrates it with the primary blepharoplast. Clear spaces are associated with internal membranes in the anterior region of the body.

(C) *Paradevescovina* Kirby 1926[9] differs from *Devescovina* in the fact that the parabasal, leaving the blepharoplast and running firstly very close to the nucleus, goes to the periphery of the body, ending in a slender rod.

Sp. typ. *P. nana* Kirby par. of *Cryptotermes hermsi* (Fig. 11).

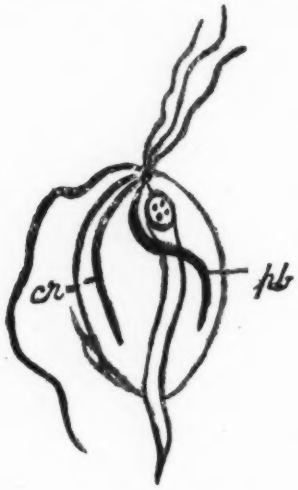

FIG. 11

Paradevescovina nana

pb, parabasal ; *cr*, cresta, much similar to the costa of Trichomonas (after Kirby)

The axostyle as being *stout*, curved, has been given as a generic character by Kirby. I think this is rather a specific character. Duboscq and Grassé[11] remark that the parabasal of *Paradevescovina* has entirely the same disposition of that of *Janickiella*, when it does not turn around the nucleus. Kirby writes[16] recently that his studies on *Fôaina gracilis* Janicki indicate that *Janickiella* Duboscq and Grassé and *Paradevescovina* Kirby are synonyms of *Fôaina*.

(D) *Pseudodevescovina* Sutherland 1933 *emend.* Kirby 1936.[17] Perfect Devescovinid in which the parabasal is constituted by a transverse band to which are attached various branches forming an accessory tuft.

Sp. typ. *P. uniflagellata* Sutherland, par. of *Kalotermes* (*Neotermes*) *insularis*, from Australia (despite its name, the flagellate possesses three anterior flagella) (Fig. 12).

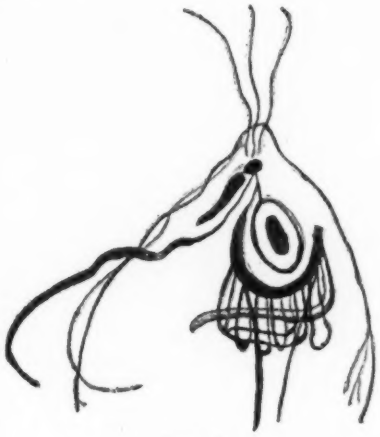

FIG. 12

Pseudodevescovina uniflagellata (after Kirby)

The differences between *Devescovina* and *Pseudodevescovina* are listed by Sutherland as: (*a*) one anterior flagellum; (*b*) absence of trailing flagellum; (*c*) different arrangement of the parabasal apparatus. Kirby[17] states that the first two points are incorrect as the flagellate possesses three anterior flagella, a trailing flagellum and a cresta which were not mentioned by Sutherland. Thus, the diagnosis of the genus given by Kirby is : Body stout, three ant. flagella, one trailing flagellum, relatively small in the known species; stout axostyle tapering to slender posterior end which may project slightly from the body ; cresta of moderate size ; parabasal apparatus large, consisting of a main limb turned transversely behind nucleus and cords attached to this; nucleus with longer axis longitudinal, with no space between membrane and chromatic mass ; small chromatin granules which are not stained deeply in iron hæmatoxyline preparations differentiated for general structures, one or sometimes two nucleoli ; in known species an investment of short spirochætes ; adherent fusiform absent.

(1) *P. uniflagellata* : 52–95, average 73 microns long ; 26–60, average 41 broad ; ant. flagella 30 ; trailing a little longer and little stouter, that only in its middle portion ; cresta 11–20, average 16 ; axostylar capitulum expanded with a more or less cylindrical appendage extending posteriorly from the portion in the papilla on the right side of the nucleus; axostyle projecting from the body ; parabasal-comprises a L- or C-shaped main limb, to the transverse part of which are attached from 7 to 19 cords, 25–30 microns long ; nucleus 7·3–12·5 average 9·8 long × 4·2–7·3 9·8 average 5·6; dense investment of short spirochætes about 8–10 microns long.

(2) *P. ramosa* Kirby 1938, par. of *Kalotermes s. lato* from Tanganyika and Mt. Meru. Length 40–113, av. 76 microns. Width 27–60, av. 42. Flagella, specially the trailer, shorter than the body, except in small individuals. Trailing flagellum slender. Cresta 5·3–7, av. 6·5. Parabasal composed of : (1) long main limb, partly chromophyll, passing more or less transversely after bending in U around one end of the nucleus ; (2) a short curved branch arising near the bend of

the U ; (3) a voluminous branched part that arised in 1–3 trunks close together near the bend of the U and subdivides at various points to produce 9–16 cords which wind rather closely around the axostyle, just posterior to the nucleus. Axostyle with projecting end and capitulum considerably expanded. Nucleus with no space between the membrane and the finely granular chromatin with one or two peripheral nucleoli. Characteristic elongated granules present just under surface all over the body. Spirochætes, many 7–8 microns long, abundant on entire surface (Fig. 13).

FIG. 13

Pseudodevescovina ramosa (after Kirby)

(E) *Caduceia* França 1918.[18] Three anterior flagella, often difficult to be distinguished. Trailing flagellum very slender, generally shorter than the body. Cresta small (Kirby), absent (Grassé). Parabasal in loose turns around the axostyle and often composed of multiple or ramified filaments. Axostyle with a large capitulum where the nucleus seems to be lodged. Pseudostriation of the ectosarc due to juxtaposition of baciliform or spirochætal organisms.

Sp. typ. *C. theobromæ* França, par. of *Neotermes gestroi* from the Island of St. Thomé, in the Atlantic (Fig. 14).

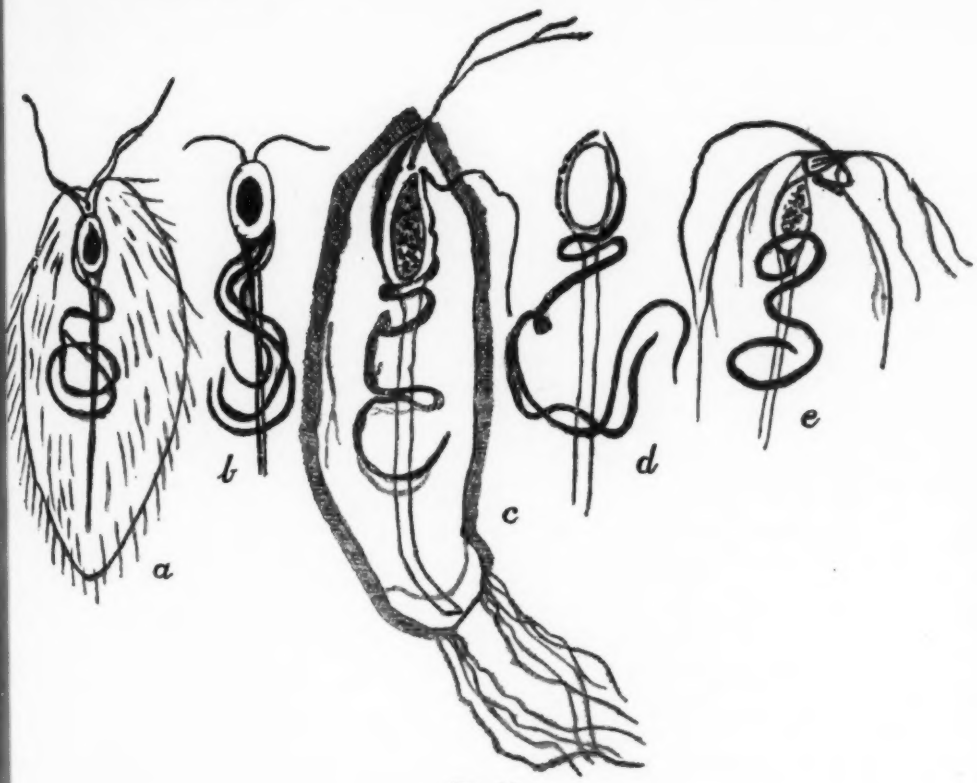

FIG. 14

Caduceia theobromæ

a, *b* (after França) ; *c*, *d* (after Grassé) ; *e* (after Kirby)

Koidzumi,[19] Cleveland, Duboscq and Grassé and Bernstein believed *Caduceia* to be synonymous of *Devescovina*. In 1922, in a discussion which I had personally with França, when we met together in Loanda (Angola) for the first Congress of Tropical Medicine, having pointed out the resemblance between *Caduceia* and *Devescovina*, França himself agreed with this synonymia and so I also adopted the same synonymia.[20] In 1935 Grassé[21] accepted the validity of the genus *Caduceia* in a paper presented to the XII International Congress of Zoology held at Lisbon. The parasite is harboured in the intestine of *Neotermes aburiensis* (?) from Ivory Coast and measures circa 100 microns × 30–35. Three very slender anterior flagella, closely united and forming a kind of pincel (França has not been able to see the dissociation of this pincel and has described therefore only one anterior flagellum). Nucleus pyriform, slightly bent in arch with a net of chromatin as in metazoan cells and with one nucleolus. Capitulum of axostyle expanded and lodging the nucleus just as a spoon lodging an egg. Trailing flagellum starting from a special basal granule, often attached to another, which gives rise to a fibril running along the flagellar base and homologous to the *costa* of *Trichomonas* and the *cresta* of *Devescovina*. Parabasal starting from another granule, and turning around the axostyle, at first close to the ventral face of the nucleus, forming then a sudden curve at right angle and running around the axostyle in loose, generally 4–5 coils. Often two or even three parabasals, parallel one to another.

Kirby[22] states definitely that " it is necessary to retain *Caduceia* as separate genus". He gives the following diagnosis ; Devescovinids of large size (known sspp. 42-124 microns, average 62-90 long), stout, bluntly pointed or rounded ends. Cresta small (1-12 microns). Three ant. flagella. Trailing flagellum a slender cord, usually less than body length. Chromatic shield composed of small granules, present against one side of the nucleus. Parabasal body single, well developed, usually coiled tightly, 3–15 turns (in *C. theobromæ* more loosely coiled). Capitulum of axostyle a simple expansion, trunk tapered to a filamentous posterior portion usually entirely enclosed in cytoplasm. Nucleus elongated longitudinally as in *Devescovina*, with well-defined membrane, peripheral clear space and coarsely granular chromatin mass. Adherent, slender rod-shaped micro-organisms producing apparent striation on restricted areas of the surface of some species, absent on others. All known species with an investment of short spirochætes, 4–6 microns long, on most of the surface.

As sspp. Kirby gives :

(1) *C. theobromæ* França, par. of *Neotermes gestri* and of *Kalotermes* (*sensu lato*) from East Africa, Mt. Meru, Moshe in Tanganyika ; Taveta Forest Kenya ; Near Rutchuru in Belgian Congo. Length 71–124, av. 90 microns. Width 38–69, av. 48. Three ant. flagella. Trailing flagellum usually a slender cord, usually shorter than the body. Cresta 8–12, av. 9·5. Parabasal body, generally spiralled in broad, loose, uneven coils, number of turns 2–5, av. 3. Chromatic shield of small deeply staining granules applied to nuclear membrane. Nucleus 15·5–18 × 6–8, av. 17 × 7·2. Slender rod-shaped micro-organisms adherent to a restricted area at the post. end. Slender spirochætes, often 4–6 microns long, forming a dense coat over the rest of the surface.

These attached spirochætes were misinterpreted by França as flagella and the parasite on this account included among *Hypermastigina*.

As special organels Kirby points out a small papilla, whose maintenance is aided by a lamella which stains deeply with iron-hæmatoxylin, in connection with the group of the three ant. flagella. The cresta was wrongly regarded by França as a broadened base of the flagellum. Branching of the parabasal occurs in a small percentage of the flagellates.

(2) *C. nova* (Grassi) syn. *Devescovina nova*, par. of *Kalotermes* (*Neotermes*) *erythraeus* from Erythrea. As Grassi stated that he had poor material and consequently gave a brief description, Kirby redescribes the parasite.[17] Length 64–144, av. 88 microns. Width 32–67, av. 47. Trailing flagellum comparatively short and slender. Cresta 9–12, av. 10·5. Axostylar capitulum simple, extended conspicuously into papilla. Trunk of axostyle tapering to filament, completely enclosed in cytoplasm. Nucleus with broad space between membrane and chromatin mass 12–17, av. 15 × 6–9, av. 7·5. Parabasal apparatus with main limb long, turned in a variable, usually wide loop around the axostyle, behind the nucleus, then in long loose spiral often of about two turns ; one to six cords, of calibre equal to main limb and directed parallel to it, attached posterior to first loop. Body invested with a coat of short spirochætes ; area near post. end of most specimens with fusiform.

(3) *C. pruvoti* (Duboscq and Grassé) syn. *Devescovina pruvoti* (*proparte*), as in their description " they evidently confused species of Tri-*cercomitus*, *Fôaina*, *Devescovina* and *Caduceia* (Kirby)."

Kirby writes,[22] " Five sspp., of *Caduceia* have been distinguished by the writer in material he has studied. Of these *C. nova* and *C. theobromæ* have been described. A sixth sp. is *C. pruvoti*, a new combination for part of the species *Devescovina pruvoti* described by Duboscq and Grassé."

I have not unhappily come across with the description of the remaining 3 sspp.

(F) *Macrotrichomonas* Grassi 1917, *syn. Gigantomonas* Connell 1932, *nec* Dogiel. Trailing flagellum band-like, usually one and half times longer than the body. Cresta having the form of a large membrane. Parabasal in tight coils. Axostyle without tapering end. No pseudostriation, despite the adherence of spiral organisms.

Sp. typ. *M. pulchra* Grassi, par. of *Glyptotermes parvulus* (Fig. 15).

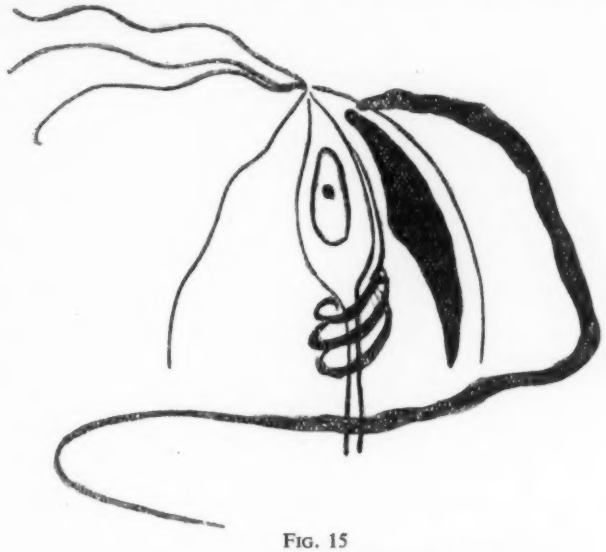

FIG. 15

Macrotrichomonas pulchra (after Grassi)

The original description of Grassi has been supplemented and corrected by Kirby[22] after the study of *M. pulchra* from different hosts which are : *Glyptotermes parvulus* from Goald Coast, *Kalotermes contracticornis* from Costa Rica, *Glyptotermes montanus* from Java, and *Glyptotermes Ceylonicus* from Ceylon. Kirby has also studied one slide from Grassi. " It may be surprising says Kirby, that the same species occurs in the African, Central American and Javan termites, but a careful comparison shows no difference except in size."

(1) *Diagnosis of Macrotrichomonas given by Kirby*. Devescovinids of large size, known sspp., 36–91 microns long, av. 52–68. Distance from the ant. end to nucleus varies, of value for taxonomic purposes. Three ant. flagella. Trailing flagellum well developed, broad band formed in some, usually 1½ times length of the body. Cresta developed into a broad internal membrane 25–49 long or more. Parabasal body usually unbranched, tightly coiled around axostyle 1–13 times. Capitulum moderately developed. Trunk of axostyle stout, tapering but not reduced to filament as in *Caduceia* and *Devescovina*, no terminal enlargement, end generally pointed, usually projecting a short distance. Nucleus of *Devescovina* type. No longitudinal rod-shaped microrganisms. Long spirochætes present on anterior and posteior parts in some species.

Diagnosis of M. pulchra given by Kirby. Dimensions of body : from *G. parvulus*. Microns 44–91, av. 67 × 21–41, av. 32 ; from *K. contracticornis* 41–82, av. 57 × 16–33, av. 25 ; from *G. montanus* 36–68, av. 52 × 19–39, av. 26. Distance from ant. end to nucleus usually 5–9, av. 7·8. Trailing flagellum a broad band, max. 1,8, part proximal to body surface more deeply staining. Length of cresta 25–35, antero-medial edge about 14–18, postero-medial 17–26, max. width 7–11. Antero-medial edge bordered by a *paracrestal* filament which continues posteriorly inside the coils of the parabasal body. No marked thickenings of cresta. Parabasal body coiled tightly around axostyle, 2–13 turns, usually 3–4. Anterior lamella. Suspensory *lamella*. Well defined bent rhizoplast. Nucleus: from that of *G. parvulus* 9·2–10·7 × 6·8–9, av. 9·6 × 7·7; of *K. contracticornis* 7·5–10·5 × 7·5, av. 8·3 × 6·4. Clear zone under membrane frequently evident, spirochætes 15–20 long, sometimes present on ant. and post. ends.

Some interesting points in Kirby's description: (*a*) the three long ant. flagella pass along the ant. border of the small papilla in two roots, the ant. of which is stouter than the other ; (*b*) on the ant. end a lot of independent granules, in connection with the flagella, parabasal cresta, etc. (In fact, the neuromotor system of these flagellates,as I have seen in many of them, is composed of physiologically independent basal granules, sometimes united or embedded one into another and often becoming anatomically separated, only in mitotic stages) ; (*c*) the side of the trailing flagellum which is adjacent to the surface of the body stains deeply with iron-hæmatoxylin to a variable breadth of about a third or half of the flagellum, which appears in this way as a clear membrane bordered by a stained filament-like edge; thus the resemblance to an undulating membrane with costa and axoneme is striking, but it is merely a superficial resemblance. The organel homologous to *costa* is the *cresta* ; (*d*) parallel and close to, but not against the antero medial border of the cresta is the *paracrestal filament*; very slender in its ant. part, thickens near the angle of the cresta and continues inside the coils of the parabasal body, usually for a distance of two or three turns. It was not seen in *M. pulchra* from *Glyptotermes ceylonicus* ; (*e*) the axostyle tapers gradually from the nucleus to its post. end which is blunt and not prolonged into a filament. A well-defined filament-like core constitutes the centre of the trunk ; (*f*) two well-defined structures extend from the ant. end of the body to contact with the nuclear membrane : (1) the *suspensory lamella*, a band passing from the region of the ant. granules to the lateral nuclear membrane, deeply staining in side view, a heavy filament apparently lying peripheral to the capitulum of the axostyle and comparable to the suspensory lamella described by Janicki, in *D. hawaiensis*—; (2) the rhizoplast not straight. but somewhat sinuous, passing from the most post. and largest of the granules to the ant. nuclear membrane; (*g*) several specimens showed the parabasal branched at the beginning of the coiled part, the branch being usually much shorter than the main limb (Fig. 16).

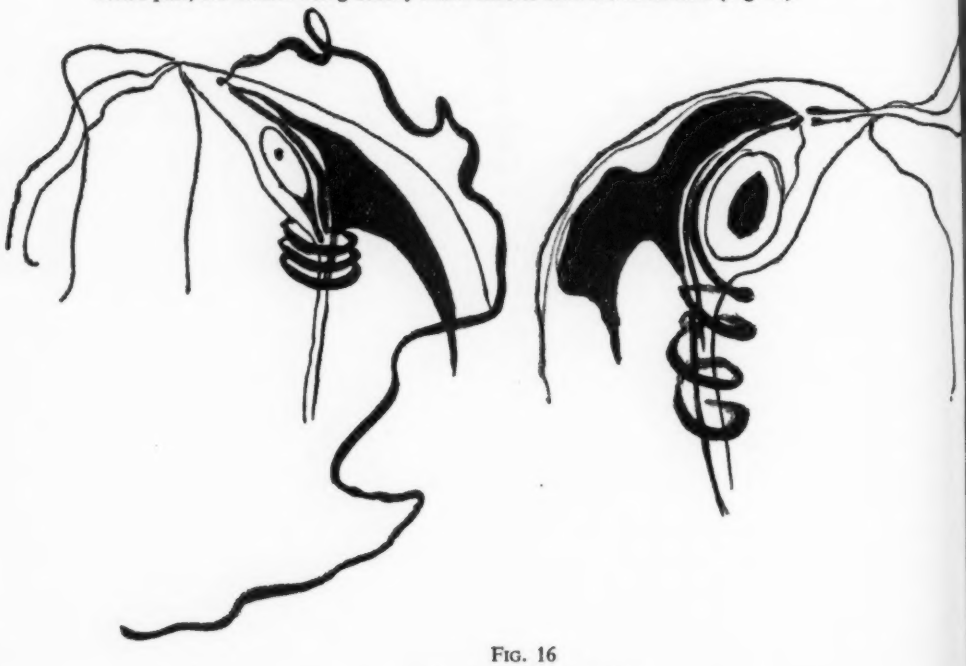

FIG. 16

Macrotrichomonas pulchra

(*a*) from *Glyptotermes ceylonicus* ; (*b*) from *Kalotermes contracticornis* (after Kirby)

(2) *M. lighti* (Connell) syn. *Gigantomonas lighti* Connell 1932, par. of *Kalotermes* (*Paraneotermes*) *simplicicornis* [Univ. of Calif. Public in Zoology 37, F. H. Connell. *Gigantomonas lighti* sp. nov. a trichomonad flagellate from *Kalotermes* (*Paraneotermes*) *simplicicornis* Bank].

I have not been able to consult this paper and therefore cannot but enumerate only this species. The name *Gigantomonas* Connell, should not be confused with *Gigantomonas* Dogiel, sp. typ. *G. herculea* Dogiel, a curious trichomonad par. of *Hodotermes massambicus*.

In writing the names of the termites I have copied the names *ipsis verbis* from the original papers. So, *Calotermes* in Grassi is *Kalotermes* in other authors, *Neotermes gestroi* in França is *N. gestri* in Kirby.

(G) *Fôaina* Janicki 1915. Three anterior flagella, one trailing flagellum. Long axostyle protruding through the posterior pole. Parabasal composed of two independent masses surrounding the nucleus.

Sp. typ. *F. gracilis* Janicki, par. of *Neotermes connexus* (Fig. 17).

FIG. 17

Fôaina gracilis (after Janicki)

I have some doubt in including the genus *Fôaina* among the *Devescovinidæ* and rather believe that it should constitute the type genus of a new family *Fôainidæ*, but having not seen such a type of flagellate, prefer to keep on this point a prudent reserve. Some opinion will be however quoted here. Kirby writes (12, page 202): "The writer's studies of *Fôaina gracilis* indicate that *Janickiella* Duboscq and Grassé and *Paradevescovina* Kirby are synonyms of *Fôaina*."

Duboscq and Grassé whose ideas on transmutation forms in the evolutive cycle of these different species are well known, however not having the support of other protozoologists, write (11, pp. 433–34) that the young stages of *D. hilli* resemble *Fôaina*. They have an analogous form, but normally do not possess but one single parabasal with three or four chromophob spherules.

Having the chance of examining many specimens in predivisional phase they have seen that they do not differ at all from *Fôaina*, such as figured by Janicki: two parabasals provenient of splittine of a pre-existent parabasal were attached to the daughter blepharoplast. They occupied differeng positions, converging to the blepharoplast, still very close to one another or near the poles of the nucleus, or superposed in such a way that it gave a perfect image of *Fôaina*. The authors believe that they represent a phase in the cycle of *D. hilli*, that Janicki has probably seen these small forms in mitotic phase, which generally occur at the same time in these flagellates and if their hypothesis is accepted, the genus *Fôaina* should be discarded from the nomenclature.

(H) *Parajoenia* Janicki 1911 *emend* Kirby 1937.[23] Moderately large size, rounded anteriorly and posteriorly, papilla not prominent. Three anterior flagella. One long trailing flagellum. Stout axostyle, usually with pointed end projecting from the body and expanded capitulum. Parabasal apparatus of two parts, a proximal, V-shaped element attached at the apex to the blepharoplast, a distal element attached to the other part near its posterior end. Nucleus with longer axis oblique or transverse, no clear space under membrane, chromatin granules small, one or more nucleoli present.

Sp. typ. *P. grassii* Janicki, par. of *K.* (*Neotermes*) *connexus*, from Hawaii, incorrectly determined as *Calotermes castaneus*.

Diagnosis of P. Grassii after Kirby. Length : microns 25–59, av. 45. Width 12–33, av. 24. Cord-like trailing flagellum 0·3 width, often 1½ times body length. Cresta 7·5–11, exceptionally 14, av. 9. Two parts of the parabasal of similar size, parabasal filament specially prominent in the distal part, in which it extends freely for some distance beyond post. end ; short filament also extending into cytoplasm from post. element of the parabasal, at its bend or anterior to the middle. Trunk of axostyle stout, post. end sharpened and projecting from cytoplasm ; capitulum broad with a high, narrow ridge or keel. Nucleus 5–6·3 × 2·3–3·4 av. 5·6 × 3. Numerous spirochætes, 15–20 long, adherent to ant. and post. parts of the body, those of post. part attached over rounded, elongate, peripheral corpuscles (Fig. 18).

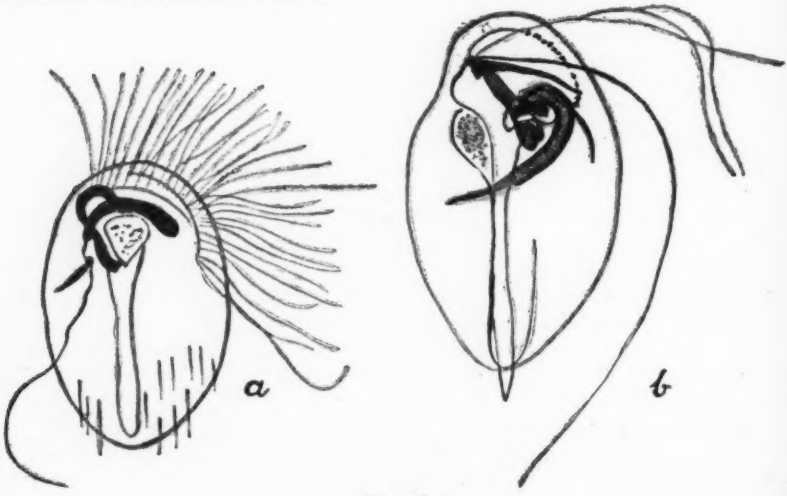

FIG. 18

Parajoenia grassi

a, (after Janicki) ; *b*, (after Kirby) the investment of spirochætes not figured.

Some remarks about Parajoenia grassii. According to Kirby, as in other Devescovinids, the flagella arise in a small anteroventral depression. Anterior to this is a blunty pointed papilla along which to its ends the three long delicate flagella are adherent. The blepharoplast is composed of four or five closely aggregated granules. The axostyle is anteriorly expanded in the right side of the nucleus into a large capitulum which occupies a good part of the anterior region of the body. From the capitulum and the ant. part of the trunk there rises, on the right side, a high narrow ridge which extends to the nucleus. In it or on it there is a layer of abundant small chromatic granules. Janicki described the axostyle as fibrillar, Kirby observed nothing like the numerous fine fibrils figured by Janicki. The axostyle possesses a membranous sheet and within it an axial part which appears quite homogeneous and dark; in the anterior of its interior part, however, there sometimes appears a darker line. The parabasal apparatus consists of two apparently separate parts, one proximal V- or L-shaped, the second distal, curved or even L-shaped. So, the parabasal is not really double, but the second part is an appendage of the first. In a few specimens, the post. ends of one or both portions of the parabasal apparatus were partly branched into two parts and in one the end of the distal portion was branched into several parts. The flagella figured by Janicki in the anterior part are adherent spirochætes and the bristle-like flagella on the post. third are adherent bacteria or spirochætes.

(I) The genera *Gigantomonas* Dogiel 1916, sp. typ. *G. herculea*, par. of *Hodotermes mossambicus* and *Janickiella* Luboscq and Grassé 1923, sp. typ. *J. grassii*, par. of *Calotermes flavicollis*, which Kirby included among the *Devescovinidæ*, the first definitely, the second rather doubtfully, find their better place among the *Trichomonadidæ*.

The systematic position of this family should therefore be the following:

Phylum	..	*Protozoa* von Siebold
Subphylum	..	*Plasmodroma* Doflein
Class	..	*Mastigophora* Diesing
Sub-class	..	*Zoomastigophora* Calkins
Order	..	*Monadidea* Butschli
Tribe	..	*Polymastigina* Blochman
Family	..	*Devescovinidæ* Poche
Genera	..	as described in the paper.

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STUDIES IN *DOLICHOS LABLAB* (ROXB.) AND (L.) THE INDIAN FIELD AND GARDEN BEAN—IV

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Seed Coat Colour Patterns

In Part II* of these studies it has been recorded that there are four seed coat colours in *lablab* namely black, chocolate, khaki and buff, and that in black, chocolate and khaki, the colour may be manifested over the whole of the seed coat or be localised, the buff colour always existing in *wholeness*. It was noted that a basic factor K is necessary for the production of the colours black, chocolate and khaki and by itself this factor K produces the *Micropylar colour*. A factor B_f produces the buff seed coat colour and is capable of converting the *Micropylar colour* into a *Whole colour*. These two factors K and B_f are absolutely linked. In Part III† it was stated that in the seed coat colours black, chocolate and khaki, several kinds of localisations could exist as distinct heritable types. The present paper deals with the description and inheritance of these seed coat colour patterns.

There are five patterns representing different stages in the manifestation of pigment on the seed coat, from a mere spot on the micropyle to very nearly covering the entire seed coat. In these patterns the background colour is white and another colour which forms the pattern is distributed over this background colour symmetrically about the hilum (Fig. 1). In dealing with these patterns the factors inducing the patterns are alone discussed. The genes for a particular pattern being present they could manifest themselves in any of the three colours black, chocolate or khaki. Since it was found that all the five patterns could exist in each of the three seed coat colours black, chocolate or khaki, in the data presented, distinctions

* Rangaswami Ayyangar, G. N., and K. Kunhi Krishnan Nambiar, *Proc. Ind. Acad. Sci.*, 1935, **2**, 74.

† Rangaswami Ayyangar, G. N., *et al.*, *Ibid.*, 1936, **4**, 411.

between these three colours have been omitted. The five patterns are described below:—

1. *Micropylar colour.*—In this pattern the colour starts as a spot on the micropyle, edges both sides of the raphe, joining again at the caruncle. From the caruncle there is a spur of colour ending in a fork. Below this fork there is an area of dotted colour which is broader towards the fork.

2. *Caruncular patch.*—When the caruncular spot and the forked spur get accentuated in colour, it results in the next pattern designated "*Caruncular patch*". In this pattern there is a patch of colour concentrated round about the caruncle. Below this patch there is an area of dotted colour as in the *Micropylar colour* pattern.

3. *Dorsal patch.*—In this pattern there is a patch of colour over the dorsal half of the seed which starts from the caruncle and proceeding towards the micropylar area stops short of it. There is a clear white area along the raphe.

4. *Marbled.*—In this the whole seed coat is interspersed with white and coloured irregular patches.

5. *Eye.*—Here the whole of the seed coat is coloured except an eye-shaped patch just around the micropyle. This white patch may sometimes extend upwards along the raphe.

The interaction of the following five factors produces the different seed coat patterns described above.

(1) *Factor* K.—This is the basic factor for the production of the colours black, chocolate and khaki on any part of the seed coat. By itself, this factor produces colour at the micropylar and caruncular zones.

(2) *Factor* C_p.—With the factor K, this produces a patch of colour over the caruncle resulting in the pattern *Caruncular patch.*

(3) *Factor* D_p.—With the factors K and C_p, this factor produces the *Dorsal patch* pattern. D_p has visible effect only in the presence of C_p.

(4) *Factor* M.—With the factors K and C_p, this factor produces the *Marbled* pattern. M also has visible effect only in the presence of C_p.

When all these three factors C_p, D_p and M are present along with the basic colour factor K, we get the next pattern, the *Eye.*

(5) *Factor* B_f.—Factor for *Whole colour*. By itself this factor produces a buff seed coat. In conjunction with the factors for any of the patterns B_f produces a *Whole colour*.

The interrelationships of these five seed coat colour patterns were studied by making suitable crosses. These are summarised and presented in the following pages, to illustrate their genetic behaviour.

In a cross between a *Micropylar colour* type and the *Caruncular patch*, the F_1 was the *Caruncular patch*. In the F_2 this segregated into *Caruncular patch* and *Micropylar colour* in the ratio of 3 : 1. A factor C_p in the presence of the factor K (*Micropylar colour* factor) gives rise to the pattern *Caruncular patch* (*vide* Table I).

TABLE I

Pure for K

Micropylar colour (c_pc_p) × *Caruncular patch* (C_pC_p)

F_1—*Caruncular patch* (C_pc_p)

F_2 Family No.	Caruncular patch	Micropylar colour
D.L. 1570	47	15
„ 1572	7	4
„ 1573	24	9
„ 1575	27	11
Total ..	105	39
Expected, 3 : 1 ratio ..	108	36

$P > 0{\cdot}5$

In a cross of another *Micropylar colour* type with the *Caruncular patch*, the F_1 obtained was the pattern *Dorsal patch*. In the F_2 this segregated into *Dorsal patch*, *Caruncular patch* and *Micropylar colour* in the ratio of 9 : 3 : 4. A factor D_p acting with C_p and K gives the pattern *Dorsal patch*. This factor is operative only in the presence of C_p. It has no visible effect with c_p (*vide* Table II).

TABLE II

Pure for K

Micropylar colour ($c_\rho c_\rho$ $d_\rho d_\rho$) × *Dorsal patch* ($C_\rho C_\rho$ $D_\rho D_\rho$)

F_1—*Dorsal patch* ($C_\rho c_\rho$ $D_\rho d_\rho$)

F_2 Family No.	Dorsal patch	Caruncular patch	Micropylar colour
D.L. 1524	47	17	22
„ 1525	46	16	18
„ 1526	37	10	14
„ 1527	24	8	11
„ 1529	49	19	26
„ 1530	18	8	12
„ 1532	27	9	11
Total ..	248	87	114
Expected, 9 : 3 : 4 ratio ..	252·54	84·18	112·24

$P > 0·5$

Simple segregations for *Dorsal patch*: *Caruncular patch* and *Dorsal patch*: *Micropylar colour* are given in Tables III and IV.

TABLE III

Pure for K *and* C_ρ

Caruncular patch ($d_\rho d_\rho$) × *Dorasl patch* ($D_\rho D_\rho$)

F_1—*Dorsal patch* ($D_\rho d_\rho$)

F_2 Family No.	Dorsal patch	Caruncular patch
D.L. 1281	54	18
„ 1282	109	33
„ 1283	227	63
„ 1284	104	38
Total ..	494	152
Expected, 3 : 1 ratio ..	484·50	161·50

$P > 0·3$

TABLE IV

Pure for K *and* D_p

Micropylar colour (c_pc_p) × *Dorsal patch* (C_pC_p)

F_1—*Dorsal patch* (C_pc_p)

F_2 Family No.	Dorsal patch	Micropylar colour
D.L. 1085	156	47
„ 1086	141	48
„ 1111	262	78
„ 1112	156	61
Total ..	715	234
Expected, 3 : 1 ratio ..	711·75	237·25

P > 0·8

A factor M with the co-operation of the factors K and C_p produces the *Marbled* pattern (*vide* Table V).

TABLE V

Pure for K, C_p *and* d_p

Caruncular patch (mm) × *Marbled* (MM)

F_1—*Marbled* (Mm)

F_2 Family No.	Marbled	Caruncular patch
D.L. 1280	91	28
„ 1305	134	53
„ 1306	222	61
„ 1307	205	63
Total ..	652	205
Expected, 3 : 1 ratio ..	642·75	214·25

P > 0·3

The factor M has no visible effect except in the presence of C_p. With c_p only the *Micropylar colour* pattern is produced (*vide* Table VI).

TABLE VI

Pure for K, d_p *and* M

Micropylar colour (c_pc_p) × *Marbled* (C_pC_p)

F_1—*Marbled* (C_pc_p)

F_2 Family No.	Marbled	Micropylar colour
D.L. 1295	55	20
„ 1296	98	29
„ 1297	85	20
„ 1298	73	24
Total ..	311	93
Expected, 3 : 1 ratio ..	303	101

P > 0·3

A recessive *Micropylar colour* pattern of the constitution KK c_pc_p, d_pd_p MM from one of the above families (D.L. 1298) was crossed with a *Caruncular patch* and produced the *Marbled* pattern in the F_1.

When a *Dorsal patch* was crossed with a *Marbled*, the *Eye* pattern was obtained in the F_1. In the F_2 this segregated into *Eye*, *Dorsal patch Marbled* and *Caruncular patch* in the ratio of 9 : 3 : 3 : 1. Thus, factors D_p and M in the presence of K and C_p give the *Eye* pattern (*vide* Table VII).

TABLE VII

Pure for K *and* C_p

Dorsal patch (D_pD_p mm) × *Marbled* (d_pd_p MM)

F_1—*Eye* (D_pd_p Mm)

F_2 Family No.	Eye	Marbled	Dorsal patch	Caruncular patch
D.L. 1083	477	155	134	49
„ 1277	43	12	14	4
Total ..	520	167	148	53
Expected, 9 : 3 : 3 : 1 ratio ..	499·5	166·5	166·5	55·5

P >0·3

Simple segregations of *Eye*: *Marbled* and *Eye*: *Dorsal patch* are given in Tables VIII and IX.

TABLE VIII

Pure for K, C_p *and* M
Marbled (d_pd_p) × *Eye* (D_pD_p)
F_1—*Eye* (D_pd_p)

F_2 Family No.	Eye	Marbled
D.L. 868	15	4
,, 896	10	4
,, 897	21	5
,, 1272	86	26
,, 1273	19	4
,, 1276	107	35
,, 1278	139	45
,, 1293	98	35
Total ..	495	158
Expected, 3 : 1 ratio ..	489·75	163·25

$P > 0·5$

TABLE IX

Pure for K, C_p *and* D_p
Dorsal patch (mm) × *Eye* (MM)
F_1—*Eye* (Mm)

F_2 Family No.	Eye	Dorsal patch
D.L. 1087	196	56
,, 1088	174	58
,, 1113	194	60
,, 1114	163	58
Total ..	727	232
Expected, 3 : 1 ratio ..	719·25	239·75

$P > 0·5$

The factors D_p and M are both inoperative without C_p (*vide* Table X). Thus C_p is essential for the production of the patterns *Eye*, *Marbled*, *Dorsal patch* and *Caruncular patch*.

TABLE X

Pure for K, D_p *and* M

Micropylar colour (c_pc_p) × *Eye* (C_pC_p)

F_1—*Eye* (C_pc_p)

F_2 Family No.	Eye	Micropylar colour
D.L. 1109	343	111
,, 1285	81	25
,, 1289	58	20
Total ..	482	156
Expected, 3 : 1 ratio ..	478·5	159·5

P > 0·7

It was shown that when a *Micropylar colour* pattern is crossed with a *Buff*, a *Whole colour* is produced in the F_1, and this segregates in the F_2 into *Whole*: *Micropylar colour*: *Buff* in a 2 : 1 : 1 ratio (due to the absolute linkage between the factors Kb_f and kB_f). It will be seen from Tables XI–XIV that the B_f factor is capable of extending each one of the other patterns into a *Whole colour*. When any of these is crossed with the *Buff* a *Whole colour* is produced in the F_1, which segregates in the F_2 into *Whole colour*, *pattern* and *Buff* in a 2 : 1 : 1 ratio.

TABLE XI

Pure for C_p, D_p *and* m

Dorsal patch (KK b_fb_f) × *Buff* (kk B_fB_f)

F_1—*Whole colour* (Kk B_fb_f)

F_2 Family No.	Whole colour	Dorsal patch	Buff
D.L. 1299	38	18	11
,, 1302	111	57	58
,, 1346	16	6	11
,, 1347	45	23	20
,, 1349	20	7	6
,, 1350	41	20	20
Total ..	271	131	126
Expected, 2 : 1 : 1 ratio ..	264	132	132

P > 0·3

TABLE XII

Pure for C_p, d_p *and* M

Marbled (KK $b_f b_f$) × *Buff* (kk $B_f B_f$)

F_1—*Whole colour* (Kk $B_f b_f$)

F_2 Family No.	Whole colour	Marbled	Buff
D.L. 890	6	3	4
Expected, 2 : 1 : 1 ratio ..	6·50	3·25	3·25

P > 0·3

TABLE XIII

Pure for C_p, D_p *and* M

Eye (KK $b_f b_f$) × *Buff* (kk $B_f B_f$)

F_1—*Whole colour* (Kk $B_f b_f$)

F_2 Family No.	Whole colour	Eye	Buff
D.L. 895	25	12	12
,, 1317	27	16	15
,, 1319	106	52	49
,, 1377	19	10	7
,, 1378	15	8	7
Total ..	192	98	90
Expected, 2 : 1 : 1 ratio ..	190	95	95

P > 0·8

TABLE XIV

Pure for C_p, d_p *and* m

Caruncular patch (KK $b_f b_f$) × *Buff* (kk $B_f B_f$)

F_1—*Whole colour* (Kk $B_f b_f$)

Note.—The F_2 of the above cross was unfortunately not grown.

In the preceding segregations the *Whole colour* had the genetic constitution Kk $B_f b_f$ and hence was always heterozygous. When the *Whole colour* has the constitution KK $B_f B_f$ it breeds true for wholeness of colour.

In the various patterns the factor B_f is absent. When the factor B_f is introduced, these patterns get converted into a *Whole colour*. In the F_2 these segregate for the B_f factor alone thereby giving rise to 3 : 1 segregation of *Whole colour*: *pattern*. Such segregations are given in Tables XV–XVIII.

TABLE XV

Pure for K, C_p, d_p *and* m

Caruncular patch ($b_f b_f$) × *Whole colour* ($B_f b_f$)

F_1—*Whole colour* ($B_f b_f$)

F_2 Family No.	Whole colour	Caruncular patch
D.L. 1363	140	46
„ 1370	43	15
„ 1460	37	10
„ 1461	32	13
Total ..	252	84
Expected, 3 : 1 ratio ..	252	84

TABLE XVI

Pure for K, C_p, D_p *and* m

Dorsal patch ($b_f b_f$) × *Whole colour* ($B_f B_f$)

F_1—*Whole colour* ($B_f b_f$)

F_2 Family No.	Whole colour	Dorsal patch
D.L. 1116	191	66
„ 1117	143	39
„ 1118	88	30
„ 1120	182	58
„ 1457	36	12
„ 1459	32	11
Total ..	672	216
Expected, 3 : 1 ratio ..	666	222

$P > 0{\cdot}50$

TABLE XVII

Pure for K, C_{ρ}, d_{ρ} *and* M

Marbled ($b_f b_f$) × *Whole colour* ($B_f B_f$)

F_1—*Whole colour* ($B_f b_f$)

F_2 Family No.	Whole colour	Marbled
D.L. 1482	82	23
„ 1485	103	41
„ 1488	75	34
„ 1493	55	13
Total ..	315	111
Expected, 3 : 1 ratio ..	319·50	106·50

P > 0·5

TABLE XVIII

Pure for K, C_{ρ}, D_{ρ} *and* M

Eye ($b_f b_f$) × *Whole colour* ($B_f B_f$)

F_1—*Whole colour* ($B_f b_f$)

F_2 Family No.	Whole colour	Eye
D.L. 693	51	24
„ 1455	105	38
„ 1486	70	17
„ 1491	50	17
„ 1513	106	34
„ 1515	87	31
„ 1516	174	56
Total ..	643	217
Expected, 3 : 1 ratio ..	645	215

P > 0·8

The following families segregate for the factors D_ρ and B_f, the factors K, C_ρ and M being pure. In this case segregations of *Whole colour*: *Eye*: *Marbled* in the ratio of 12:3:1 are obtained (*vide* Table XIX).

TABLE XIX

Pure for K, C_ρ *and* M

Marbled ($d_\rho d_\rho b_f b_f$) × *Whole colour* ($D_\rho D_\rho B_f B_f$)

F_1—*Whole colour* ($D_\rho d_\rho B_f b_f$)

F_2 Family No.	Whole colour	Eye	Marbled
D.L. 1436	407	102	37
„ 1483	64	15	4
„ 1486	51	17	7
„ 1487	44	13	3
Total ..	566	147	51
Expected, 12:3:1 ratio ..	573·00	143·25	47·75

P > 0·8

In the following families segregations for the factors D_ρ and B_f occur, the factors K, C_ρ and m being pure. Here also a ratio of 12:3:1 is obtained between *Whole colour*, *Dorsal patch* and *Caruncular patch* (*vide* Table XX).

TABLE XX

Pure for K, C_ρ *and* m

Caruncular patch ($d_\rho d_\rho b_f b_f$) × *Whole colour* ($D_\rho D_\rho B_f B_f$)

F_1— *Whole colour* ($D_\rho d_\rho B_f b_f$)

F_2 Family No.	Whole colour	Dorsal patch	Caruncular patch
D.L. 1304	156	33	8
„ 1314	356	86	36
„ 1315	45	12	2
„ 1508	124	32	8
„ 1509	37	8	5
Total ..	718	171	59
Expected, 12:3:1 ratio ..	711·00	177·75	59·25

P > 0·8

The following families segregate for the factors C_p and D_p, the factors K, M and b_f being pure. A 9 : 3 : 4 ratio of *Eye*: *Marbled*: *Micropylar colour* is obtained (*vide* Table XXI).

TABLE XXI

Pure for K, M *and* b_f

Micropylar colour (c_pc_p D_pD_p) × *Marbled* (C_pC_p d_pd_p)

F_1—*Eye* (C_pc_p D_pd_p)

F_2 Family No.	Eye	Marbled	Micropylar colour
D.L. 1290	98	42	47
,, 1291	135	30	50
,, 1292	121	39	47
,, 1294	66	25	33
Total ..	420	136	177
Expected, 9 : 3 : 4 ratio ..	412·29	137·43	183·24

$P > 0.7$

From the data presented it will be seen that in the seed coat colour patterns the following genotypes are possible.

Micropylar colour ..	KK c_pc_p d_pd_p mm b_fb_f
	KK c_pc_p D_pD_p mm b_fb_f
	KK c_pc_p d_pd_p MM b_fb_f
	KK c_pc_p D_pD_p MM b_fb_f
Caruncular patch ..	KK C_pC_p d_pd_p mm b_fb_f
Dorsal patch ..	KK C_pC_p D_pD_p mm b_fb_f
Marbled ..	KK C_pC_p d_pd_p MM b_fb_f
Eye ..	KK C_pC_p D_pD_p MM b_fb_f
Whole colour ..	KK c_pc_p d_pd_p mm B_fB_f
	KK C_pC_p d_pd_p mm B_fB_f
	KK c_pc_p D_pD_p mm B_fB_f
	KK c_pc_p d_pd_p MM B_fB_f
	KK C_pC_p D_pD_p mm B_fB_f
	KK C_pC_p d_pd_p MM B_fB_f
	KK c_pc_p D_pD_p MM B_fB_f
	KK C_pC_p D_pD_p MM B_fB_f

Speckling

The patterns so far considered consist of localisations of a single colour over different regions of the seed coat. There is another kind of pattern in which the colour is broken up into a number of specks over a background colour of khaki (Fig. 2). This phenomenon has been termed *Speckling*. *Speckling* can exist both in the *Whole colour* and in all the five patterns mentioned before. In these patterns the *Speckling* is confined to the area normally pigmented in them, the rest of the area remaining white. Black and chocolate *Speckling* have been met with, the background colour in both being khaki. Khaki *Speckling* is not met with probably because when it occurs it is indistinguishable from the background colour which is also khaki.

From the experiences presented below it may be assumed that a factor S_p produces the solid colour. Its allelomorph s_p is responsible for *Speckling*. S_p has visible effect only in the presence of the factors for the chocolate and black seed coat colours.

In the following families segregations between *Whole colour* and *Speckled whole colour* occur. These represent the segregations for the factor S_p, the factors K and B_f being pure (*vide* Table XXII).

TABLE XXII

Pure for K *and* B_f

Speckled whole colour ($s_p s_p$) × *Whole colour* ($S_p S_p$)

F_1—*Whole colour* ($S_p s_p$)

F_2 Family No.	Whole colour	Speckled whole colour	
D.L. 1321	111	43	In black seed coat
,, 1322	150	42	do.
,, 1323	88	27	do.
,, 1324	68	24	do.
,, 1325	103	32	do.
Total ..	520	168	
Expected, 3 : 1 ratio ..	516·00	172·00	

$P > 0{\cdot}7$

The following families segregate for the factor S_p, factors K, C_p, D_p and m being pure. Simple segregations of *Dorsal patch* to *Speckled Dorsal patch* occur in this case (*vide* Table XXIII).

TABLE XXIII

Pure for K, C_p, D_p *and* m

Speckled Dorsal patch (s_ps_p) × *Dorsal patch* (S_pS_p)

F_1—*Dorsal patch* (S_ps_p)

F_2 Family No.	Dorsal patch	Speckled Dorsal patch	
D.L. 1354	51	21	In black seed coat
„ 1355	18	10	do.
Total ..	69	31	
Expected, 3 : 1 ratio ..	75	25	

P > 0·1

The following families segregate for the factors S_p and B_f, the factors K, C_p, D_p and m being pure (*vide* Table XXIV).

TABLE XXIV

Pure for K, C_p, D_p *and* m

Dorsal patch (S_pS_p b_fb_f) × *Speckled Whole colour* (s_ps_p B_fB_f)

F_1—*Whole colour* (S_ps_p B_fb_f)

F_2 Family No.	Whole colour	Speckled Whole colour	Dorsal patch	Speckled Dorsal patch	
D.L. 1308	81	20	29	9	In black seed coat
„ 1309	97	21	30	19	do.
„ 1310	155	50	52	18	do.
Total ..	333	91	111	46	
Expected, 9 : 3 : 3 : 1 ratio	326·79	108·93	108·93	36·31	

P > 0·1

The following families segregate for the factors S_p, D_p and B_f, the factors K, C_p and m being pure (*vide* Table XXV).

TABLE XXV

Pure for K, C_ρ *and* m

Caruncular patch ($S_\rho S_\rho$ $d_\rho d_\rho$ $b_f b_f$) × *Speckled Whole colour* ($s_\rho s_\rho$ $D_\rho D_\rho$ $B_f B_f$)

F_1—*Whole colour* ($S_\rho s_\rho$ $D_\rho d_\rho$ $B_f b_f$)

F_2 Family No.	Whole colour	Speckled Whole colour	Dorsal patch	Speckled Dorsal patch	Caruncular patch	Speckled Caruncular patch	
D.L. 1448	187	41	37	10	12	2	In black seed coat
„ 1449	107	33	16	6	6	1	do.
„ 1450	90	33	13	6	12	1	do.
„ 1451	133	64	33	14	16	5	do.
Total ..	517	171	99	36	46	9	
Expected, 36:12:9:3:3:1 ratio ..	493·92	164·64	123·48	41·16	41·16	13·72	

P > 0·1

In the following families segregations for the factors S_ρ and B_f occur in the seed coat colours black (KK PP $C_h C_h$) and chocolate (KK pp $C_h C_h$) resulting in a 27 : 9 : 9 : 9 : 3 : 3 : 3 : 1 ratio as shown below (*vide* Table XXVI).

TABLE XXVI

Pure for C_h, K, C_ρ, D_ρ *and* M

Black Eye (PP $S_\rho S_\rho$ $b_f b_f$) × *Chocolate Speckled Whole colour* (pp $s_\rho s_\rho$ $B_f B_f$)

F_1—*Black Whole colour* (Pp $S_\rho s_\rho$ $B_f b_f$)

F_2 Family No.	WHOLE COLOUR		EYE		SPECKLED WHOLE COLOUR		SPECKLED EYE	
	Black	Chocolate	Black	Chocolate	Black	Chocolate	Black	Chocolate
D.L. 1517	23	7	5	2	4	1	..	1
„ 1518	32	4	10	2	15	1	5	1
„ 1519	41	16	12	2	10	3	4	2
„ 1520	30	11	9	7	7	3	1	2
„ 1521	40	17	16	7	20	2	7	..
„ 1522	20	6	5	3	3	3	2	..
„ 1523	32	7	11	3	8	2	2	..
Total ..	218	68	68	26	67	15	21	6
Expected, 27:9:9:3:9:3:3:1 ratio ..	206·31	68·77	68·77	22·92	68·77	22·92	22·92	7·64

P > 0·7

The following family segregates for the factors S_p and B_f in conjunction with segregations for the seed coat colours black (KK PP C_hC_h) and khaki (KK PP c_hc_h). It was not possible to distinguish *Speckling* in the khaki seed coat colour. The numbers obtained agree with the theoretical expectation if we assume that khaki *Speckling* cannot be made out from khaki solid colour.

TABLE XXVII

Pure for K *and* P

Black Speckled Whole colour (C_hC_h s_ps_p B_fB_f) × *Khaki micropylar colour* (c_hc_h S_pS_p b_fb_f)

F_1—*Black Whole colour* (C_hc_h S_ps_p B_fb_f)

F_2 Family No.	Black				Khaki	
	Whole colour	Speckled Whole colour	Micropylar colour	Speckled Micropylar colour	Whole colour	Micropylar colour
D.L. 1430 ..	33	7	10	2	14	5
Expected, 27:9:9:3:12:4 ratio ..	29·94	9·98	9·98	3·33	13·31	4·44

P > 0·8

When a buff was crossed with the *Speckled Whole* seed coat, a solid *Whole colour* was produced in the F_1. In the F_2, this segregated into *Whole*, *Speckled Whole* and *Buff* in the ratio of 9 : 3 : 4 (*vide* Table XXVIII).

TABLE XXVIII

Pure for B_f, P *and* C_h

Black Speckled Whole colour (KK s_ps_p) × *Buff* (kk S_pS_p)

F_1—*Black Whole colour* (Kk S_ps_p)

F_2 Family No.	Black Whole colour	Black Speckled Whole colour	Buff
D.L. 1442	117	51	54
,, 1498	32	11	16
,, 1499	32	11	14
Total ..	181	73	84
Expected, 9 : 3 : 4 ratio ..	190·13	63·38	85·50

P > 0·3

From one of the above families (D.L. 1442), selections of *Buff* and *Speckled Whole colour* were carried forward to the next generation. All the *Buffs* bred true while some of the *Speckled Whole colour* selections segregated according to expectation into *Speckled Whole colour* and *Buff* in a 3 : 1 ratio (*vide* Table XXIX).

TABLE XXIX

Pure for P, C_k, B_f *and* s_ρ

Buff (kk × *Black Speckled Whole colour* (KK)

F_1—*Black Speckled Whole colour* (Kk)

F_2 Family No.	Black Speckled Whole colour	Buff
D.L. 1500	31	9
„ 1503	32	9
„ 1558	20	5
„ 1559	28	9
„ 1560	21	8
Total ..	132	40
Expected, 3 : 1 ratio ..	129	43

$P > 0{\cdot}5$

The factor s_ρ has thus no visible effect on the buff seed coat also.

Summary

1. In the seed coat colours black, chocolate and khaki, five heritable types of localisations are met with namely, the *Micropylar colour*, the *Caruncular patch*, the *Dorsal patch*, the *Marbled* and the *Eye* patterns.

2. These patterns arise by the interaction of the five factors K, C_ρ, D_ρ, M and B_f. By itself, the factor K produces the *Micropylar colour*. K being present, the association of one or more of three factors C_ρ, D_ρ and M, produces the various patterns. Thus, with the C_ρ factor the pattern *Caruncular patch* is produced. In the presence of K and C_ρ, the factor D_ρ produces the *Dorsal patch* while the factor M produces the *Marbled* pattern. The combined presence of K, C_ρ, D_ρ and M results in the *Eye* pattern.

3. The factor B_f which by itself produces a buff seed coat is capable of converting any of the above patterns into a *Whole colour*. Factors K and B_f are absolutely linked.

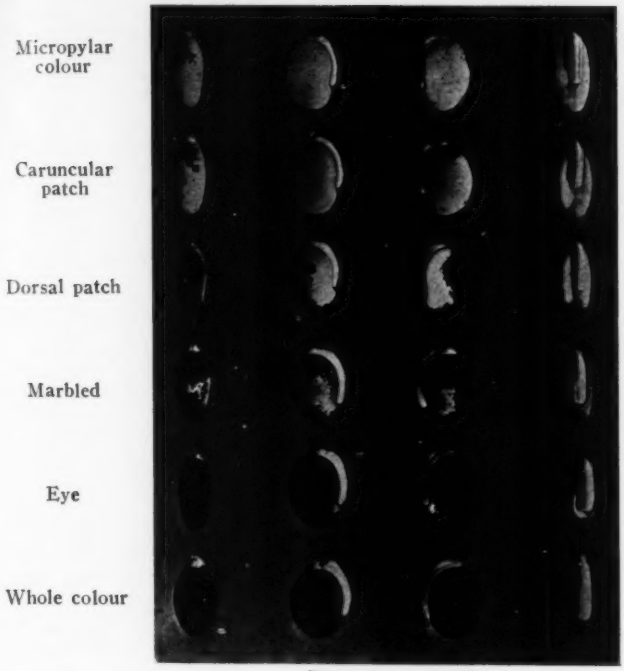

FIG. 1

Speckled seeds
×3

FIG. 2

4. A factor S_p produces the smooth and even distribution of colour over the seed coat. Its allelomorph s_p is responsible for *Speckling*. Thus with K and B_f, S_p produces a solid *Whole colour*, while s_p results in the *Speckled Whole colour*. The factors K, b_f, and s_p are necessary for the production of each of the *Speckled patterns*. *Speckling* can manifest itself in only two colours black and chocolate and is not perceptible when it occurs on a khaki or buff background.

STUDIES ON THE HISTOLOGY AND COLOURATION OF THE PERICARP OF THE SORGHUM GRAIN

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The sorghum grain is a caryopsis. It is oval to subspherical. The embryo is fairly large and is placed at the less convex surface and more towards the base. It is acentric so that externally it is opposed to the pericarp and internally surrounded by the endosperm. The side which bears the embryo is flat and from the tip of the embryo it converges to the stylar base (Fig. 1)

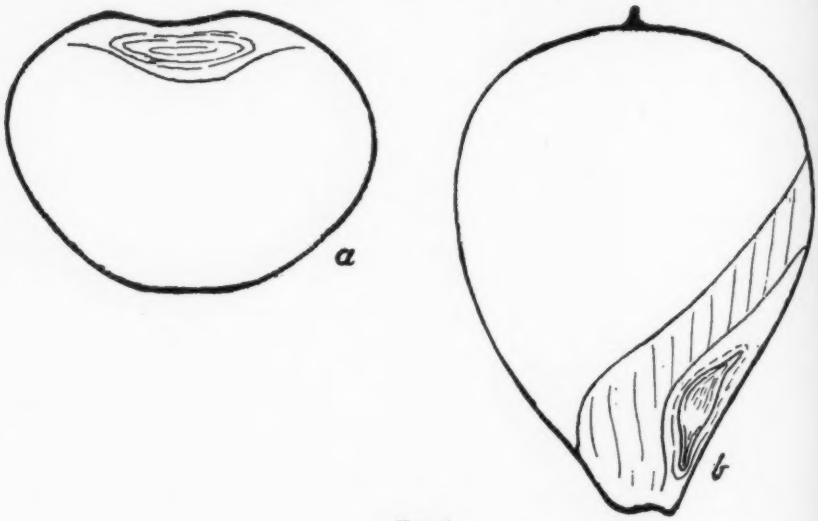

Fig. 1

Organogeny of the pericarp (Figs. 2–10)

Ovaries from the time of pollination upto the endosperm formation were fixed in Navashin's fluid and the later stages in Carnoy's, at successive stages of growth. Sections were stained in either Gentian violet-iodine or Hæmatoxylin. Types with a 'Nucellus' (Feterita) and without 'Nucellus' (A.S. 29 Periamanjal) were used in these studies.

The early stages of development were observed to be the same in both types. A young ovary is oval with two stigmatic branches typical in gramineæ. The ovule is anatropus to almost campylotropus. The funicle is broad and short, consequently the seed coat becomes unequal in length on either side of the micropyle. There are two integuments. The micropyle is formed by the inner integument, the outer one stopping far short of it. The ingteguments are only two layers wide but may increase in width at the chalazal end where the seed coats fit into the notch formed by the fusion of the carpel wall to form the stigmatic branches.

The nucellus is cellular, thin walled and fills up the whole of the ovule. A small beak is also found. The embryo-sac is rather small for the size of the grain and is, placed somewhat deep in the nucellus. The antipodals form a tissue of 10–12 cells.

After fertilization the endosperm nuclei form a small number of free nuclei in the neighbourhood of the zygote. These form a cellular tissue by the time the zygote has undergone the second division. The endosperm thus formed is found mostly along the periphery of the embryo-sac so that a hollow tissue is formed. The expansion of the embryo-sac is very gradual. Even in a week-old grain the nucellus would not have been completely absorbed. However, in a mature grain there is no nucellus left, the absorption being complete. The deposition of starch begins in about ten days after fertilization. The aleurone is differentiated last.

The pericarp or the ovary wall is intensely green in the young stages and consists of several layers of cells. The epidermis contains a thin cutin and the walls are unthickened. The cells are rectangular in transverse sections. Immediately below this is a layer of cells which are slightly larger-lumened than the other cells. This is the hypodermis. After this is the mesocarp tissue of 9–10 layers. The innermost layers are of smaller lumen than the other mesocarp cells. There is an inner epidermis, the cells of which are very small and have no cutin. These cells towards maturity develop thickenings, become cylindrical and form the " tube cells " of the American authors (*vide infra*). The mesocarp and the hypoderm are chlorophyllous in young ovaries. The pericarp keeps pace in growth along with the embryo. The green colour is lost gradually only towards the setting in of the starch grains in the endosperm. Permanent starch may be stored in the mesocarp cells. With the maturity of the grains about 2½–3 weeks after fertilization, the chlorophyll is completely lost.

The integuments, as already mentioned are two. The outer one remains rudimentary and begins to disorganise very early, but remnants of it,

however, persist till even after the endosperm formation. The cells are narrow, rectangular and narrower than broad. The inner integument on the other hand grows with the developing grain. The inner layer has rectangular, more or less isodiametrical cells while the outer is longer than broad. Both layers grow in size till the formation of the endosperm, thereafter the outer layer begins to gradually disorganise. The inner layer on the other hand even at the time of the fertilization shows much larger tissue than the outer layers and grows much more in size than the outer one with the growth of the grain. The growth is most evident at the chalazal end. Towards maturity this layer in some grains becomes brown and hyaline, while in others it disorganises at maturity. Evidently it was this layer that was described as ' nucellus ' by some American authors (*vide infra*). The behaviour of this layer in mature grains is more fully described later.

Mature pericarp.—1. The sorghum caryopsis has the pericarp fused with the seed. Winton (1902) described the structure of this sorghum caryopsis as made up of 5 layers in the pericarp, *viz.*, (i) epidermis, (ii) hypodermis, (iii) mesocarp, (iv) cross cells, (v) tube cells, and a ' nucellus ' layer. In the endosperm he mentioned the aleurone and the starch cells.

Swanson (1928) mentions the same layers as Winton. He notes also the absence of the ' nucellar layer ' in some types. This layer is otherwise called the ' hyaline layer ' by him, and it is considered to be morphologically derived from the nucellus. He notes also that this layer when present is coloured brown. The mesocarp is starchy and it masks the brown of the nucellus, but when it is vestigeal the brown colour of the nucellus is perceptible through the mesocarp. He has also measured the several tissues of the pericarp and nucellus in 18 types of grain sorghums.

Hector (1936) doubts the origin of the nucellar layer of Swanson. He concludes from evidence that it is the testa (inner integument).

Harrington and Crocker (1923) studied the structure of the pericarp in Johnson grass (*S. halepense*). They describe only the inner integument and make no mention of the nucellus layer.

Materials and Methods

Since the time of Winton (*loc. cit.*), the numerous wild and cultivated races of Sorghums have been systematically classified by Snowden (1936). Advantage was taken of the large collection at the Millets Breeding Station, Coimbatore, to make a comparative study of the typical forms in each sub-series and species of Snowden's classification. In the following is presented a classified list of the species studied:—

TABLE I

Lot Number	Sub-series and Species	Local Name and Place	Grain Colour
	WILD SORGHUMS		
Eu-sorghum			
Arundinacea—(*a*)	Series Spontanea		
S. 88*a* ..	*S. sudanense*	Guntur Farm, India	Brown
S. 207 ..	*S. arundinaceum*	S. Rhodesia	do.
S. 221 ..	*S. virgatum*	Tunis grass, Texas, U.S.A.	do.
Arundinacea—(*b*)	Series Sativa		
Drummondii—			
S. 102 ..	*S. nitens*	From Uganda, through Kew	do.
A.S. 5148 ..	*S. aterrimum*	Obtained as a mixture in a sample of brown corn from Odessa, U.S.S.R.	do.
Halepensia—			
S. 164 ..	*S. halepense*	Godavari, India	do.
Para-sorghums—			
S. 96 ..	*S. versicolor*	Texas, U.S.A.	Light pearly
S. 234 ..	*S. dimidiatum*	Through Sugarcane Breeding Station, Coimbatore, India	Brown
S. 250 ..	*S. purpureo-sericeum*	Through Sugarcane Breeding Station, Coimbatore, India	do.
Non-classified—			
M.S. 4357 ..	*S. nitidum*	Rangaswamikoil, Coimbatore	do.
M.S. 4360 ..	*S. Stapfii*	Maniyachi village, Koilpatti, India	do.
	CULTIVATED SORGHUMS		
Arundinacea—(*b*)	Series Sativa		
Guineensia—			
A.S. 683 ..	*S. margaritiferum var. ovulifer*	N. Provinces, Sierra Leone	White pearly
A.S. 4548 ..	*S. guineense*	Tiv from Yandev, Nigeria	White chalky
A.S. 4681 ..	*S. conspicuum* var. *conspicuum*	Kimungampombo, Sanger Dt., Tanganyika	do.
A.S. 403 ..	*S. Roxburghii* var. *hians*	Talai Virichan Cholam, Coimbatore	do.
A.S. 1093 ..	*do.*	do.	do.

TABLE I—*Contd.*

Lot Number	Sub-series and Species	Local Name and Place	Grain Colour
A.S. 1092 ..	*S. Roxburghii* var. *hians*	Talai Virichan Cholam, Coimbatore	White chalky
A.S. 1055 ..	*do.*	do.	White pearly
A.S. 3819 ..	*do.*	Jenora Josia, Patna Dt., Bihar, India	do. (violet)
A.S. 1008 ..	*do.*	Khed Jonna, Vizagapatam, India	Yellow
Nervosa—			
A.S. 3783 ..	*S. membranaceum*	Adhroo Jowar, C. Provinces, India	Pink pearly
A.S. 5219 ..	*S. nervosum*	Yishien Shantian, Peiping, China	Light brown
A.S. 5812 ..	*S. melaleucum*	Red durra, S. Africa	Reddish-brown
A.S. 210 ..	*S. splendidum* var. *splendidum*	Jonna, Harivanam, Bellary, India	White pearly
Bicoloria—			
A.S. 5195 ..	*S. dochna*	Stock feed, Kaoliang, China	Brown
A.S. 3356 ..	*S. bicolor*	Goose-neck sorghum, U.S.A.	do.
A.S. 3815 ..	*S. miliiforme* var. *rotundulum*	Juar, Bihar and Orissa, India	Reddish-brown
A.S. 4599 ..	*S. elegans*	Jebere, Tanganyika	White chalky
Caffra—			
A.S. 4202 ..	*S. coriaceum*	Juriba, N. Rhodesia	Reddish-brown
A.S. 4413 ..	*S. caffrorum*	Early Kalo Sorghum, Colorado U.S.A.	Pink
A.S. 3464 ..	*do.*	Wonder, F.C. 8986, Texas	White chalky with brown nucellus
A.S. 3998 ..	*S. nigricans*	Karaviru, Bukoba and Behrambo, Tanganyika	Reddish-brown
A.S. 4660 ..	*do.*	Kikuma. Tanganyika	do.
A.S. 5186 ..	*S. caudatum*	Feterita, Sudan	White chalky
A.S. 4564 ..	*S. caudatum* var. *faterita*	Kwarbiya, Sokoto, Nigeria	do.
Durra—			
A.S. 29 ..	*S. durra*	Periamanjal Cholam, Coimbatore, India	Yellow, brown-wash

TABLE I—*Contd.*

Lot Number	Sub-series and Species	Local Name and Place	Grain Colour
A.S. 1098 ..	*S. durra*	Periamanjal Cholam, Coimbatore, India	Yellow, brown-wash
A.S. 1842 ..	*do.*	do.	do.
A.S. 2635 ..	*do.*	do.	do. (no brown-wash)
A.S. 2613 ..	*do.*	From Periamanjal Cholam crass	Brown
A.S. 1958/3 ..	*do.*	(Ears throwing brown grain mutants)	White
T. 1 ..	*S. cernuum*	Tella Jonna, Bellary, India	White pearly
A.S. 171 ..	*do.*	do.	do.
A.S. 151 ..	*S. subglabrescens*	Sakkaraguligai Jonna, Bellary, India	do.
A.S. 195 ..	*do.*	Periavellai, Coimbatore, India	White chalky
A.S. 367 ..	*do.*	Vellai cholam, Dindigul, India	White pearly, no nucellus
A.S. 389 ..	*do.*	Sen cholam, Manapparai, India	Light red
A.S. 809 ..	*do.*	Chinnamanjal Coimbatore, India	Yellow
A.S. 3672 ..	*do.*	Manjakattai cholam, Dindigul India	White pearly, red-base, red wash

The grains were soaked in water to render them soft. Free-hand sections were cut and mounted in glycerine. The measurements were done with a Zeiss X 7 ocular and X 40 objective combination. A linear micrometer was used. The data presented in the Tables (*vide infra*) represent averages of ten readings taken at random. Photographs were done with a Beck's ¼-plate camera.

Description of the tissues

In both the wild and cultivated sorghums, the grain colour was found to dissolve to some extent in water so that the water is coloured yellow, brown or red according to the grain. With white grains the water becomes yellow tinged.

(*a*) *Wild sorghum* (Figs. 11 and 12–14)

The wild sorghum grains are characterised by the persistence of the integument in all of them and the vestigeal nature of the mesocarp. The

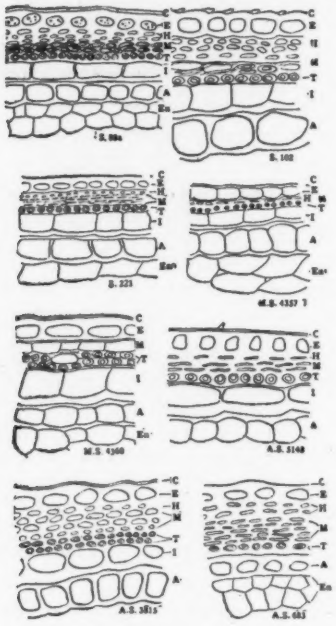

FIG. 11

pericarp is made up of (i) a very conspicuous epidermis, (ii) Hypodermis, (iii) Mesocarp (vestigeal), (iv) tube cells, and (v) conspicuous integument. S. 96 has the simplest structure. The pericarp here consists of only epidermis, tube-cells and integument.

(i) The outer epidermis is one-layered. There is always a layer of cutin. The cells are oval to rectangular in transverse sections. The outer walls are generally thickened and the lumen small, but in S. 96 and S. 221, they are not much thickened and have a larger lumen. In A.S. 5148 the radial walls are much thickened. S. 234 resembles S. 96 but the contour of the grain depends on the size of the thickness of the integument. The cells are generally devoid of contents. In S. 207 the contents of the cells have a yellowish colour while in S. 250 and S. 88, they are coloured brown.

(ii) The hypoderm is generally single layered. The cells are usually much thickened so that the lumen becomes very small (almost obliterated as in A.S. 5148). The layer in extreme cases as in S. 250 and S. 221 becomes fused with the inner wall of the epidermis. It is absent in S. 96, S. 234 and M.S. 4357. The cells are generally of a uniform size but may be varying as in S. 102. It is discontinuous in S. 250 and M.S. 4360. The

cells usually are devoid of contents. The layer is commonly coloured brown or yellowish brown; in S. 207 and S. 102 the walls are yellowish.

(iii) *Mesocarp.*—This layer is generally absent in the typical wild sorghums (S. 96 and M.S. 4357). However, there is a small tissue in S. 88 *a* and S. 164. The cells are crushed and are non-starchy. In S. 207 and S. 221 it is starchy and 4–5-layered. The layer may be discontinuous and isolated mesocarp cells occur as in S. 250, S. 102 and M.S. 4360. The cells are thin-walled when starchy, otherwise they are thickened and sometimes crushed together, so that the lumen is completely obliterated as in A.S. 5148. The cells are sometimes coloured yellowish or brown especially when they are crushed together. They may have contents similarly coloured.

(iv) *Tube cells.*—This layer as already pointed out is formed mainly from the inner epidermis of the pericarp. The layer is constant in all types. This tissue is easily identified in sections by the ring-shaped, thick-walled, cells. In types like S. 96, S. 234 and M.S. 4357 the outer epidermis abuts directly on the tube cells. Very often this layer is found fused with the integument so that in many cases the cells appear as blunt protrusions of the integument. The tissue is generally 1–2-layered; cells small and coloured brown.

(v) The integument is a very constant tissue appearing next to the tube cells. It is generally composed of uniform, single-layered, large lumened cells. The cells are mostly thickened on the inner wall and to a slighter extent along the outer, while the radial walls are not thickened at all. The tissue becomes slightly wider along the chalazal and micropylar ends. In S. 234 and S. 102 the width of the tissue is very varying. S. 164 shows the widest integument in all sorghums. The cell walls in this tissue are usually coloured deep brown or yellowish brown. The cells are filled up with a homogeneous matter brown in colour. Harrington and Crocker (*loc. cit.*) find that this is tannic in nature. It may also be often devoid of contents.

A typical wild Sorghum is thus made up of a fairly massive epidermis. A hypodermal layer fused with the epidermis and usually very difficult of differentiation is found. Thereafter occurs a layer of tube cells and a very massive integumental layer. A small mesocrap may be present or may be completely absent. The wild sorghums show a uniform brown pigment in all the layers. The pigmentation deposition as well as the size of the several tissues has been given in Tables II and III.

TABLE II

Size of the Pericarp layers

Sample No.	Grain Colour	Epidermis (*a*)	Hypoderm (*b*)	Mesocarp (*c*)	Tube cells (*d*)	Integument	Total Pericarp	Aleurone
Arundinacea—		μ	μ	μ	μ	μ	μ	μ
S. 221 ..	Brown	(*a*+*b*+*c*) =	11·6		3·8	9·6	25·0	13·6
S. 88*a* ..	do.	8·0	5·8	6·0	5·6	12·0	37·4	14·4
S. 207 ..	do.	12·0	4·2	25·2	4·8	12·8	59·0	12·0
Drummondii—								
A.S. 5148 ..	do.	(*a*+*b*) =	16·2	16·2	4·48	33·0	69·88	20·4
S. 102 ..	do.	(*a*+*b*) =	14·4	6·0	10·0	7·4	37·8	15·2
Halepense—								
S. 164 ..	do.	7·6	4·4	5·2	3·2	48·4	68·8	8·8
Para-sorghums—								
S. 96 ..	Light pearly	(*a*+*b*+*d*) =	18·4	nil		6·8	25·2	13·8
S. 234 ..	Brown	(*a*+*b*+*d*) =	10·4	nil		10·0	20·4	15·8
S. 250 ..	do.	(*a*+*b*) =	10·8	10·8	4·8	22·0	48·4	16·0
Non-classified— ..								
M.S. 4357 ..	do.	(*a*+*b*+*d*) =	12·0	nil		6·2	18·2	16·0
M.S. 4360 ..	do.	12·0	6·8	nil	10·0	24·4	53·2	16·4

M.S. 4360 ..	do.	12·0	6·8	nil	10·0	24·4	53·2	16·4
Guineense—									
A.S. 683 ..	White pearly	8·0	4·4	14·4	4·0	nil	30·8	12·8
A.S. 4548 ..	White chalky	9·6	9·0	108·8	4·4	nil	131·8	25·6
A.S. 4681 ..	do.	8·6	20·0	76·0	4·0	nil	108·6	24·0
A.S. 403 ..	do.	11·2	11·2	88·4	6·8	nil	117·6	14·6
A.S. 1093 ..	do.	10·0	8·4	57·6	4·0	nil	80·0	19·2
A.S. 1902 ..	do.	8·6	7·2	57·6	4·6	nil	78·0	9·6
A.S. 1055 ..	White pearly	8·8	8·8	43·6	4·0	nil	65·2	11·0
A.S. 3819 ..	White violet	13·2	12·2	26·0	7·4	28·0	86·8	18·0
A.S. 1008 ..	Yellow	8·6	6·4	58·0	7·0	17·6	97·6	9·6
Nervosa—								
A.S. 3783 ..	Pink pearly	15·0	20·0	64·0	8·4	nil	107·4	18·4
A.S. 5219 ..	Light brown	15·6	9·4	130·8	7·6	22·8	186·2	16·8
A.S. 5812 ..	Reddish-brown	13·8	21·6	118·8	10·0	nil	151·0	12·8
A.S. 210 ..	White pearly	$(a+b)$ =	19·6	30·4	4·8	nil	54·8	17·6
Bicoloria								
A.S. 5195 ..	Brown	8·2	5·2	48·0	4·0	19·6	85·0	25·2
A.S. 3356 ..	do.	12·8	7·0	29·6	5·2	24·8	79·4	11·6
A.S. 3815 ..	Reddish-brown	9·8	2·6*	14·5*	6·5	23·0	56·4	11·4
A.S. 4599 ..	White chalky	14·0	12·0	58·8	4·4	3·6	92·8	16·0
Caffra—								
A.S. 4202 ..	Reddish-brown	21·6	12·8	42·8	4·6	23·2	105·0	20·8
A.S. 4413 ..	Pink	14·0	22·0	64·0	8·0	nil	108·0	19·2

TABLE II—(*Contd.*)

Sample No.	Grain Colour	Epidermis (*a*)	Hypoderm (*b*)	Mesocarp (*c*)	Tube cells (*d*)	Integument	Total Pericarp	Aleurone
		μ	μ	μ	μ	μ	μ	μ
A.S. 3464 ..	White chalky with brown nucellus	9·4	5·0	106·4	3·8	12·0	136·6	13·0
A.S. 3998 ..	Reddish-brown	13·0	7·2	18·4	8·8	40·0	87·4	19·2
A.S. 4660 ..	do.	12·8	18·0	36·5	8·8	62·0	138·0	12·0
A.S. 5186 ..	White chalky	11·2	10·4	116·4	4·4	28·4	170·8	23·6
A.S. 4564 ..	do.	12·2	13·0	103·2	13·6	36·4	178·4	23·6
Durra—								
A.S. 29 ..	Yellow, brown-wash	13·4	14·0	61·2	8·4	nil	97·0	20·0
A.S. 1098 ..	do.	15·2	4·0	23·6	4·0	nil	46·8	14·4
A.S. 1842 ..	do.	11·4	30·2	36·4	15·6	nil	93·6	17·2
A.S. 2635 ..	Yellow, no brown-wash	14·2	25·6	31·6	6·8	nil	78·2	16·0
A.S. 2613 ..	Brown	13·2	9·6	34·4	4·0	50·4	111·6	24·4
Cernuum—								
T. 1 ..	White pearly	11·8	11·6	28·8	9·2	nil	61·4	20·4
A.S. 171 ..	do.	8·0	8·0	20·4	4·4	nil	40·8	20·0
Subglabrescens—								
A.S. 151 ..	do.	8·8	10·8	33·6	6·4	nil	59·6	10·4
A.S. 195 ..	do.	14·0	14·8	101·2	8·0	nil	138·0	21·2
A.S. 367 ..	do.	11·4	3·4	38·0	4·4	nil	57·2	13·2
A.S. 389 ..	Light-red	14·2	6·2	34·4	9·2	nil	64·0	15·2
A.S. 809 ..	Yellow	16·6	10·4	27·4	12·0	nil	66·4	18·2
A.S. 3672 ..	White pearly, red base	11·8	12·8	39·6	7·8	nil	73·0	13·2

TABLE III

A.S. 3672 ..	White pearly, red base	11·8	12·8	39·6	7·8	nil	73·0	13·2

TABLE III

Colour Deposition in the Pericarp of the Sorghum Grain

Sample No.	Seed Colour	Epidermis	Hypodermis	Mesocarp	Tube cells	Integument	Aleurone
Arundinacea—							
S. 88*a* ..	Brown	Outer wall light yellow	Light yellowish	Brownish or yellowish contents	Yellow to brown	Brown	No colour
S. 207 ..	do.	Walls yellow, contents yellowish	Walls yellowish	Walls yellowish	Brown	do.	do.
S. 221 ..	do.	Light brown	Light brown	Yellowish-brown	do.	Outer wall intense brown, inner wall pale brown	do.
Drummondii—							
A.S. 5148 ..	do.	Yellowish-brown	Yellowish-brown	do.	do.	do.	do.
S. 102 ..	do.	Yellow	Light yellow	Light yellow	Brownish-yellow	Brownish-yellow	do.
Halepensea—							
S. 164 ..	do.	Brown, outer wall reddish-brown	Walls brown	Walls brown	Brown	Brown	do.
Para-sorghums—							
S. 96 ..	Light pearly	Brown	nil	nil	do.	do.	do.
S. 250 ..	Brown	Brownish-contents	Similar to epidermis	(Not continuous layer)	do.	do.	do.
S. 234 ..	do.	do.	Not conspicuous	nil	do.	do.	do.

TABLE III—(*Contd.*)

Sample No.	Seed Colour	Epidermis	Hypodermis	Mesocarp	Tube cells	Integument	Aleurone
Non-classified—							
M.S. 4357 ..	Brown	Brown	Same as epidermis	nil	Brown	Brown	No colour
M.S. 4360 ..	do.	Walls deep brown	Brown	Brown	do.	do.	do.
Guineensea—							
A.S. 683 ..	White pearly	Yellowish	No colour	No colour	Yellowish	nil	do.
A.S. 1548 ..	White chalky	No colour	do.	do.	No colour	nil	do.
A.S. 4681 ..	do.	do.	do.	do.	do.	nil	do.
A.S. 403 ..	do.	Yellowish	Yellowish	do.	Yellowish	nil	do.
A.S. 1093 ..	do.	No colour	No colour	do.	do.	nil	do.
A.S. 1902 ..	do.	Light yellow	Light yellow	do.	Light yellow	nil	do.
A.S. 1055 ..	White pearly	Walls pale yellow	Yellowish	do.	do.	nil	do.
A.S. 3819 ..	(Violet)	Yellowish	do.	do.	Yellowish	Brown	do.
A.S. 1008 ..	Yellow	Yellowish-brown	Yellowish-brown	Yellowish	Dark brown	do.	do.
Nervosa—							
A.S. 3783 ..	Pink pearly	Pale reddish-brown with yellowish-brown contents	Pale reddish-brown	Rarely coloured	Yellowish	nil	do.
A.S. 5219 ..	Light brown	Brownish-yellow	Brownish-yellow	Wall very light yellow	Brown	Deep brown	do.

A.S. 5219 ..	Light brown	Brownish-yellow	Brownish-yellow	Wall very light yellow	Brown	Deep brown	do.
A.S. 5812 ..	Reddish-brown	Reddish-brown	Reddish-brown	No colour	Dark purple	nil	do.
A.S. 210 ..	White pearly	Yellowish contents	Same as epidermis	do.	Yellow	nil	do.
Bicoloria—							
A.S. 5195 ..	Brown	Brown	Brown	do.	Brown	Brown	do.
A.S. 3356 ..	do.	Yellowish-brown	Same as epidermis	do.	do.	Dark brown	do.
A.S. 3815 ..	Reddish-brown	Yellowish	Yellowish	Yellowish	Dark brown	Reddish-brown	do.
A.S. 4599 ..	White chalky	No colour	No colour	No colour	No colour	nil	do.
Caffra—							
A.S. 4202 ..	Reddish-brown	Reddish-brown	Reddish-brown	Light reddish-brown	Deep reddish-brown	Deep reddish-brown	do.
A.S. 4413 ..	Pink	Brown	Brown	No colour	Yellow	nil	do.
A.S. 3464 ..	White chalky with brown nucellus	No colour, occasionally light reddish-brown	Same as epidermis	do.	Light reddish-brown	Deep reddish-brown	do.
A.S. 3998 ..	Reddish-brown	Brown	Brown	Brown	Brown	Brown	do.
A.S. 4660 ..	do.	do.	do.	do.	Dark brown	do.	do.
A.S. 5186 ..	White chalky	No colour	No colour	No colour	No colour	Reddish-brown	do.
A.S. 4564 ..	do.	Very light yellow	Very light yellow	do.	do.	Yellowish-brown	do.
Durra—							
A.S. 29 ..	Yellowish-brown	Yellow	Yellow	do.	Yellow	nil	do.
A.S. 1098 ..	Yellow, brown-wash	Walls yellow, contents yellow	do.	do.	do.	nil	Walls yellowish

TABLE III—*Contd.*

Sample No.	Seed Colour	Epidermis	Hypodermis	Mesocarp	Tube cells	Integument	Aleurone
A.S. 1842 ..	Yellow, brown-wash	Walls yellow	Yellow	No colour	Yellowish-brown	nil	No colour
A.S. 2635 ..	Yellow, no brown-wash	Yellow	do.	do.	Intense yellow	nil	do.
A.S. 2613 ..	Brown	Brown	Brown	Walls brown	Blackish-brown	Yellowish-brown	do.
A.S. 1958/3 ..	do.	Brownish-yellow	Very light brown	No colour	Brownish-yellow	Brown	do.
A.S. 1958/3 ..	White grain mutant	No colour	No colour	do.	No colour	nil	do.
Cernuum—							
T. 1 ..	White pearly	Walls light yellow	Walls light yellow	do.	do.	nil	Walls very light yellow
A.S. 171 ..	do.	do.	do.	do.	Walls very light yellow	nil	No colour
Subglabrescens—							
A.S. 151 ..	do.	do.	do.	do.	do.	nil	do.
A.S. 195 ..	White chalky	No colour	No colour	do.	Yellowish	nil	do.
A.S. 367 ..	White pearly	do.	do.	do.	do.	nil	do.
A.S. 389 ..	Light red	Walls red	Light red	Sometimes red-wash, else nil	Red	nil	do.
A.S. 809 ..	Yellow	Deep yellow walls	Deep yellow	One layer only yellow	Slight yellow	nil	Slight yellowish
A.S. 3672 ..	White pearly, red-base, red wash	Light reddish-brown, in places where colour is present, otherwise no colour	Light reddish-brown, as in epidermis	No colour	Reddish-brown as in epidermis	No colour	No colour

(*b*) *Grain Sorghums* (Figs. 11 and 15–19)

Mature grains from typical varieties in each sub-order have been examined. Out of the 31 sub-orders of Snowden (*loc. cit.*) only 10 have been omitted. In some of the more important ones as in the Durra, Caffra and Roxburghii more than one sample has been examined.

The grain is made up of the same layers as the wild ones. In transverse sections the caryopsis is oval. The central portion of the grain is occupied by the white endosperm. The pericarp forms a thin lining round this white endosperm. The width of the pericarp increases towards the embryo, which is placed towards the flatter side of the grain. Very little endosperm tissue occurs between the embryo and the pericarp externally. In the longitudinal sections the pericarp increases again at both the micropylar and the chalazal ends of the grain.

(i) The epidermis is the least varying of all the layers. The cutin is generally thin and discontinuously deposited. The cells are rectangular. The thickening may vary considerably. Extremes are found in the same sub-order. Usually they are thickened on the outer and radial walls. In some cases the lumen becomes almost obliterated owing to the thickening. Nor is this character restricted to either chalky or pearly grains alone. The epidermal cells often have granular contents.

(ii) The hypoderm is usually single-layered and much thickened. The cells are usually small and the lumen narrow. The thickening is generally on all the four walls. Very often the layer becomes hard to distinguish from the thickening of the epidermal wall (A.S. 3556). It may often contain 2–3 layers of cells. Granular contents may sometimes be present. In A.S. 3464 the cells are filled with a homogeneous material.

(iii) Mesocarp is generally a parenchymatous tissue of varying numbers of layers. This tissue usually has 5–6 layers of cells but 10–14 layers of cells are not uncommon. The deposition of the starch in these layers depends upon the variety. In the same sub-order both starchy and non-starchy forms may occur. The predominantly starchy ones are usually not coloured. The non-starchy ones are either pale-yellow or brownish coloured. The outermost 2–3 layers often becomes thick-walled and non-starchy (A.S. 210, A.S. 2635 and A.S. 1902). This is more common in the pearly grains. Cases in which the innermost 2–3 layers become thickened also occur. This thickening leads to the reduction in the cell-lumen and very often the cells are found jammed together.

(iv) *Tube cells.*—This layer has elongated cylindrical cells more or less circular in cross sections. The cells are all thickened. This layer is derived

from the inner epidermis of the pericarp and a few layers immediately above it. Winton (*loc. cit.*) mentions a layer of cross cells. These are probably some of the last layers of the mesocarp cells since the only definitely different tissue next to the mesocarp is the one formed by the cylindrical tube cells. The tissue contains usually of a single layer of cells, but sometimes two to three layers may be found (A.S. 1902 and A.S. 5186). These cells are constant in all types. In A.S. 210 the cells are found to be large, whereas in A.S. 3464 they are very small. In A.S. 4564 the tissue is not continuous. Quite often the inner epidermis remains unthickened and the tube cells may be seen in different stages of formation.

(v) *Integument.*—The integument does not occur in all types. It is absent in most of the white, yellow or red grains but always found in browns. In certain white grains as in A.S. 5186 and A.S. 3819 it is present, then the grain takes on a light blue tint or violet. The cells are large and brown. Generally they are filled up with a homogeneous brown substance, so that the individual cell walls are difficult to be distinguished. The layer appears as a single continuous sheet. The inner walls are much thickened. The radial walls are very thin. The outer ones are thickened slightly. Generally there is only one layer but as in A.S. 1008 two layers may be present. This is probably both the layers of the integument persisting. In certain types the cell lumen does not contain any deposits and remains empty (A.S. 5195). The colouration becomes much diluted in such types. In some of the white grains as in A.S. 4548 and A.S. 4599 the integument is incompletely absorbed so that isolated strips of cells may be found here and there, sometimes much crushed. These cells were found to be entirely free from colouration (Fig. 18).

The sizes of the different tissues are given in the annexed Table II.

Endosperm.—The endosperm consists of (1) the starch cells which form the bulk of the tissue and (2) the aleurone cells which are specialised from the outermost layers of the endosperm.

The starch cells are hexagonal to rectangular, thin walled, large cells filled with large simple starch grains. The endosperm towards the aleurone is sometimes filled with smaller grains in addition to the large starch grains. These are similar to the aleurone grains. The layers appear horny in texture when the starch grains are compact and when less compact they form a floury endosperm.

The aleurone cells are smaller and very regular. The grains are globular and somewhat smaller than the endosperm starch grains. The largest aleurone cells (24 μ–20 μ) have been found in Caffra (A.S. 5186) and Durra

(A.S. 2613, A.S. 195, A.S. 171, A.S. 29 and T. 1) groups, while the Roxburghii (A.S. 1008 and A.S. 1902) contain some of the smallest cells (9·6 μ). In *S. halepense* the aleurone cells are only 8 μ wide (*vide* Table II).

The colouration of the sorghum grain is mainly confined to the pericarp and rarely to the aleurone. The endosperm is always devoid of colour.

On the pericarp tissues the colour when present is found invariably in the epidermis. It is rather light in the hypodermis and rather deeper in the tube cells. The mesocarp is sometimes coloured but more often not. When the mesocarp is predominantly starchy it is coloured least but when it is thickened and non-starchy it is coloured light yellow. In some of the brown grains, however, the whole mesocarp is coloured. The basic colour is yellow and on this yellow appears a light brown giving a brownish-yellow grain. The red colour of the grain appears in sections most often as light brown or yellowish-red owing to the basic yellow and also the thinness of the sections through which light is passing. The true browns and reds also have this yellow basic colour (Ayyangar *et al.*, 1933). In A.S. 3672 the grain is very light coloured. Except in the regions of the wash, the pericarp is colourless. At the region of the wash the colour is found in the epidermis, hypodermis and the tube cells. In all these regions the colour is light. It is mostly yellow with red mixture giving in this grain a cumulative effect of yellowish-brown in thin sections. Ayyangar and Nambiar (1938) have assumed a factor 'M' causing yellow or red wash, according as the base is yellow or red. This factor is best expressed with the allelomorphs 'w' and 'i'.

The integument is present in almost all the brown grains. This layer is always coloured brown. When the integument is coloured brown the tube cells also are coloured. Sometimes the tube cells are not coloured though the integument is coloured (A.S. 3464, A.S. 3819 and A.S. 5186). The epidermis and the hypodermis become coloured depending upon the extent of the brown pigmentation. In A.S. 4599 the integument is not coloured. In some instances the grain is dark brown without having the integument. This colouration confined only to the pericarp is due to the tube cells which take on a deeper, dark yellow colour (A.S. 5812 and A.S. 171).

The aleurone is very rarely coloured. It then shows a light yellow pigmentation. In such cases it is the cell wall which takes on a light yellow ground colour. The contents are rarely coloured yellow. Sometimes a dull reddish tint may also occur (A.S. 5195).

The wild sorghums are all coloured brown and show a distribution of colour in the tissues which is closely similar to the cultivated ones.

A table giving the distribution of colour in the different tissues is appended (Table III).

The size of the pericarp when considered group by group according to Snowden's classification gave no definite clue to the relationship of the families. It was on the other hand seen that within every group the same variations could occur. The epidermis and the tube cells as already noted were found to be the least variable amongst the several groups. Even the wild sorghums gave the same size variations.

The hypodermis and mesocarp on the other hand are much more variable. The size of the pericarp is more or less directly proportional to the sizes of these tissues. The hypoderm may increase by a layer or two. The mesocarp itself is very varying according to the presence or absence of the starch grains. When filled with starch the cells have wide lumen and the walls are thin. In the non-starchy grains the mesocarp is less developed and secondly the tissue is collapsed or crushed so that it becomes compact and narrow. In many grains the mesocarp may be narrow but starchy, the grains here being rather small. In many of the pearly grains this condition is common. In the predominantly starchy, chalky grains the starch grains are large and the tissue very wide. Thus the size of the pericarp becomes proportional to the size of the mesocarp tissue.

The integument is also found to be confined to no particular group. It appears both in the starchy and non-starchy grains. The presence of this layer does not much influence the size of the other layers of the pericarp and seems to be inherited as an independent one.

Of the cultivated sorghums A.S. 683 and A.S. 3815 come nearest to the wild sorghum type, but in Margaritiferum the integument is wanting (Fig. 11).

In the case of grains reported as Pseudomutations (Ayyangar *et al.*, 1939; Sieglinger, 1940), the brown grains appearing in the purely white grained panicles have a brown integument while the white grains occurring in a purely brown-grained head do not possess this tissue though the parental grains have the tissue. Grains showing striping of brown and white colours also occur. The sections of such grains showed the absence of the integument in the white portions and its presence in the coloured areas (Fig. 19).

In some of the white grain crosses the F_1 was found to be brown. The same experience was found also in crosses between (i) yellow grains with

brown-wash crossed with white chalky and (ii) yellow grains with brown-wash and yellow grains without brown-wash. The parents as well as the F_1 grains were examined for colouration. In Table IV is given the sizes of

TABLE IV

F_1s and Parents

A.S. Number	Epi-dermis	Hypo-dermis	Meso-carp	Tube-cells	Integu-ment	Total of peri-carp	Aleu-rone
171 White pearly ..	8·0	8·0	20·4	4·4	nil	40·8	20·0
1902 White chalky ..	8·6	7·2	57·6	4·6	nil	78·0	9·6
F_1 3914 Brown ..	11·2	6·4	46·8	5·6	46·8	70·0	12·6
195 White chalky ..	14·0	12·0	101·2	8·0	nil	138·0	21·2
403 do. ..	11·2	11·2	88·4	6·8	nil	117·6	14·6
F_1 5597	13·8	7·2	66·0	8·4	40·8	95·4	11·6
1098 Yellow with brown-wash	15·2	4·0	23·6	4·0	nil	46·8	14·4
403 White chalky ..	11·2	11·2	88·4	6·8	nil	117·6	14·6
F_1 5600	12·0	5·4	14·2	10·8	40·8	42·4	11·6
1842 Yellow with brown-wash	11·4	30·2	36·4	15·6	nil	93·6	17·2
2635 Yellow with no brown-wash ..	14·2	25·6	31·6	6·8	nil	78·2	16·0
F_1 3926	14·4	10·0	23·6	14·4	28·8	62·4	10·8
367 White pearly ..	11·4	3·4	38·0	4·4	nil	57·2	13·2
3464 White chalky with brown 'nucellus' ..	9·4	5·0	106·4	3·8	12·0	136·6	13·0
F_1 6515	16·4	12·0	89·6	6·8	21·2	146·0	12·8

the several tissues of the pericarp in the parents and F_1s. It was found that all the parents lacked the integument, except A.S. 3464 whereas the F_1s showed well-developed integuments (Figs. 15–17), thus showing that complementary factors are responsible for the manifestation of the integument. Vinall and Cron (1921) and Sieglinger (1924) have obtained a 9 : 7 ratio for Brown : White grains. Sieglinger (1933) has shown that Brown 'nucellus' to no Brown 'nucellus' is 3 : 1. This cross is similar to the cross A.S. 367 × A.S. 3464 examined here, but the other crosses are between

parents without integuments. Ayyangar *et al.* (1936) found an absolute linkage between blackish purple of sheath and glume and ' nucellar ' brown.

In the above crosses it was also noted that when the two parents differed widely in the number of layers of cells in the mesocarp tissues the F_1 tended to be like the parent with lower number of layers. The cells with starch grains however depend on the amount of tissue left unthickened. The size of the layer does not seem to indicate the number of layers of cells, since the width of the individual cells may vary with the amount of starch deposited.

The sorghum pericarp could be divided primarily into starchy and non-starchy mesocarp. The integument occurs irrespective of the mesocarp being starchy or not. The main colours as found in sections are—(i) yellow, (ii) brown, and (iii) red. In the white grains there is always a very light yellow colouration natural to the cell walls. The reds and browns have always a yellow background. Some of the red have a mixture of brown colour while some of the browns are reddish browns. The colour is found primarily in the epidermis, hypodermis, and the tube cells. The mesocarp is coloured when the layer is non-starchy or when the walls are thickened. Sometimes when the colouration of the other tissues is very intense the mesocarp also takes on the same colour. The integument except in two cases already mentioned are coloured brown, whenever present. Complementary factors are responsible for the occurrence of this tissue.

Summary

1. The histology of the pericarp of wild and cultivated races of sorghum have been described.

2. The organogeny of the pericarp has been studied. The tube cells were found to arise from the inner epidermis of the pericarp while the integument was from the inner integument and not from the nucellus. The latter is completely absorbed.

3. The wild sorghums are characterised by a very thin pericarp consisting of only epidermis and tube cells and sometimes a very small tissue of mesocarp. An integument is always present. All wild sorghums are brown coloured.

4. The cultivated sorghums have similar layers to the wild ones but the number of layers is greater. The grains could be distinguished into two main groups (i) starchy and (2) non-starchy. The integument is found only in some grains and absent in all others. The grains with the integument are generally brown.

5. The colour of the pericarp is found mostly in the epidermis, hypodermis and the tube cells. The integument is always coloured brown and the tube cell is also coloured similarly whenever an integument is present. The mesocarp is rarely coloured.

6. An examination of the F_1 and parents of some of the crosses revealed that while both the parents had no integuments at all, the F_1 had an integument indicating complementary factors.

7. On examination of pseudomutation grains and grain chimera it was seen that whenever there was a brown colour there was an integument present and absent when the grain was not brown.

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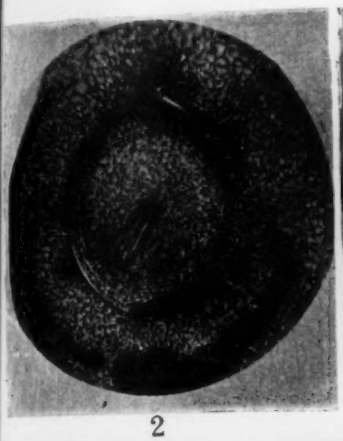
EXPLANATION OF ILLUSTRATIONS

FIG. 1. Diagrammatic. *a*, cross-section; *b*, longitudinal sections of sorghum grain. The black outer layer represents the pericarp. × about 8.

FIGS. 2–10. Organogeny of the pericarp. Reduced in reproduction by T, tube cells ; I, Integument. FIG. 2. A. S. 29 young ovule showing the embryo-sac, inner integuments and the micropyle. × 150. FIG. 3. do. Embryo-sac with the zygote, shows the micropyle formation by the inner integuments only. × 80. FIG. 4. do. Older grain showing an increase in the layer of the integuments at the place of the fusion of the pericarp. × 450. FIG. 5. do. The zygote surrounded by an endosperm tissue. The inner layer of the inner integument much enlarged. × 40. FIG. 6. do. Older grain magnified to illustrate the growth of the inner layer of the inner integument and the resorbtion of the outer integument. The inner epidermis of the pericarp is beginning to thicken. × 450. FIG. 7. do. The integument well grown. The inner epidermis of the pericarp rounding off to form the tube cells. × 450. FIG. 8. do. The inner epidermal layer thickened and formed into tube cells. × 450. FIG. 9. do. Ovary much advanced, the nucellus completely absorbed. Inner integument alone is left, cross layers not yet formed. × 300. FIG. 10. Feterita—showing the well-developed inner integument which later turns brown. Nucellus still present. × 40.

FIG. 11. Diagrammatic sketches of sorghum grain transverse sections. A.S. 3518 and A.S. 683 two cultivated sorghums coming nearest to the wild sorghums, C, cutin, E, epidermis, H, hypodermis, M, Mesocarp, E*n*, endosperms. Names of varieties found in Text-Table I.

FIGS. 12–21. Transverse sections of sorghums. Reduced in reproduction by Letterings as in Fig. 11. FIG. 12. *S. versicolor* no mesocarp, large aleurone, integument present. × 300. FIG. 13. *S. dimidiatum*, unequal development of inner integument, large aleurone. × 300. FIG. 14. *S. halepense* largest integument. × 300. FIG. 15. *S. arundinaceum*—Starchy mesocarp. × 300. FIG. 16. *S. purpureosericeum*—mesocarp discontinuous. × 300. FIG. 17. A.S. 171 Tella Jonna—white pearly. Shows general structures of typical pearly grain. Mesocarp thick-walled, narrow, non-starchy, no integument. × 300. FIG. 18. As. 1902—T. V. Cholam—white chalky. Shows the general structure of typical chalky grains. Mesocarp very broad and starchy, no integument. × 300. FIG. 19. A.S. 3915 F_1 of A.S. 1902 × A.S. 171. Integument present, mesocarp more like A.S. 171 but more starchy. × 300. FIG. 20. A.S. 4599—grain showing the colourless integument. × 250. FIG. 21. A.S. 5519/2—striped grain (brown and white) from pseudomutant heads—brown in white. Shows the development of patches of integument (*a*) and no integument (*b*).



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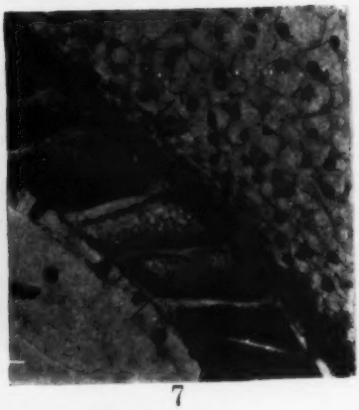

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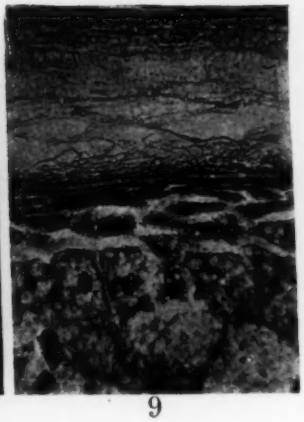

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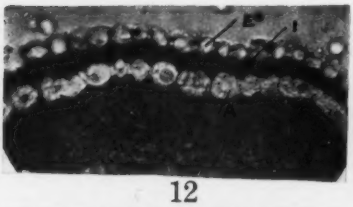

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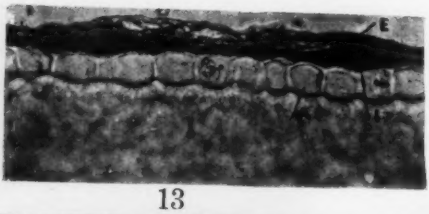

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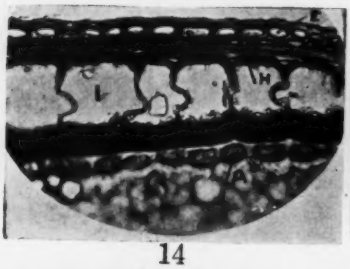

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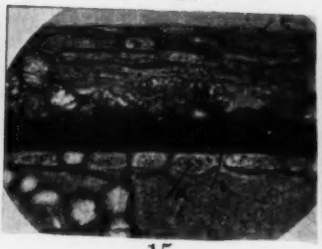

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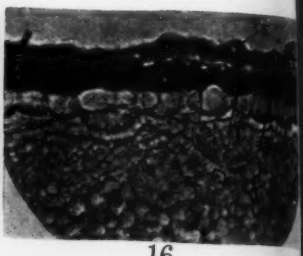

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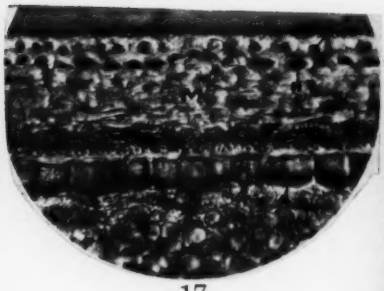

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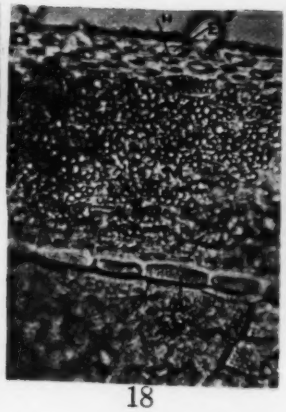

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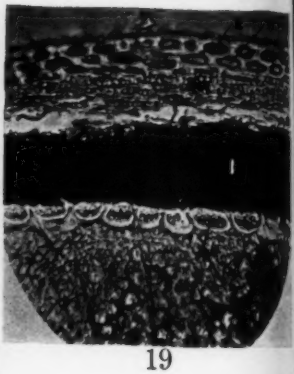

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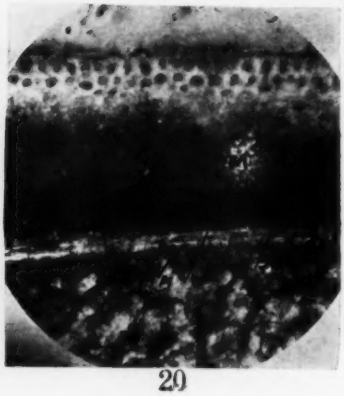

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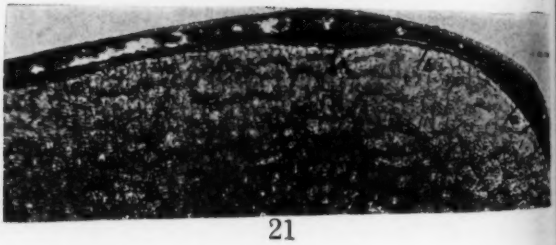

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DEVELOPMENT OF THE VERMIFORM APPENDAGE IN *GREVILLEA ROBUSTA* GUNN.

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(Communicated by Prof. A. Subba Rau)

The formation of the *vermiform appendage* of the endosperm from the lower end of the embryo-sac in the seeds of *Grevillea robusta* Cunn., a member belonging to the Proteaceæ, has already been mentioned in a previous paper published some time back (Kausik, 1938). Recently, a further detailed investigation of this remarkable structure was made with a view to determine the series of stages in its origin, growth and final fate during the development of the seed. A brief account of some of the outstanding features met with in this development is given in the following note, the details being reserved for a fuller paper to be published shortly.

The facts presented here are based on examination of a number of entire preparations, both in a living state and after killing, staining and mounting permanently according to the methods of dissection already described (Kausik, 1939), and also of microtome sections prepared in the usual way. The figures illustrating this note are not camera lucida drawings; they are diagrammatic sketches, not drawn to any definite magnification, but represented on an approximately uniform scale and showing the relative proportions, and are intended to give an idea of the succession of events met with in the development of the *appendage*.

In the living preparations the cytoplasm of the *vermiform appendage* exhibits a slow and steady streaming movement, and the nuclei are seen being carried along in this current and constantly changing their positions. As the appendage grows older this streaming movement becomes checked here and there on account of the formation of cross-walls in the appendage which thereby becomes divided into a number of compartments (Fig. 4). Later, when groups of small cells are formed in these compartments, a feature becoming more and more progressive with further development of the *appendage*, the cytoplasmic movement stops almost completely.

Regarding the origin and growth of the *vermiform appendage*, examination of a number of dissected out preparations, constituting a progressive

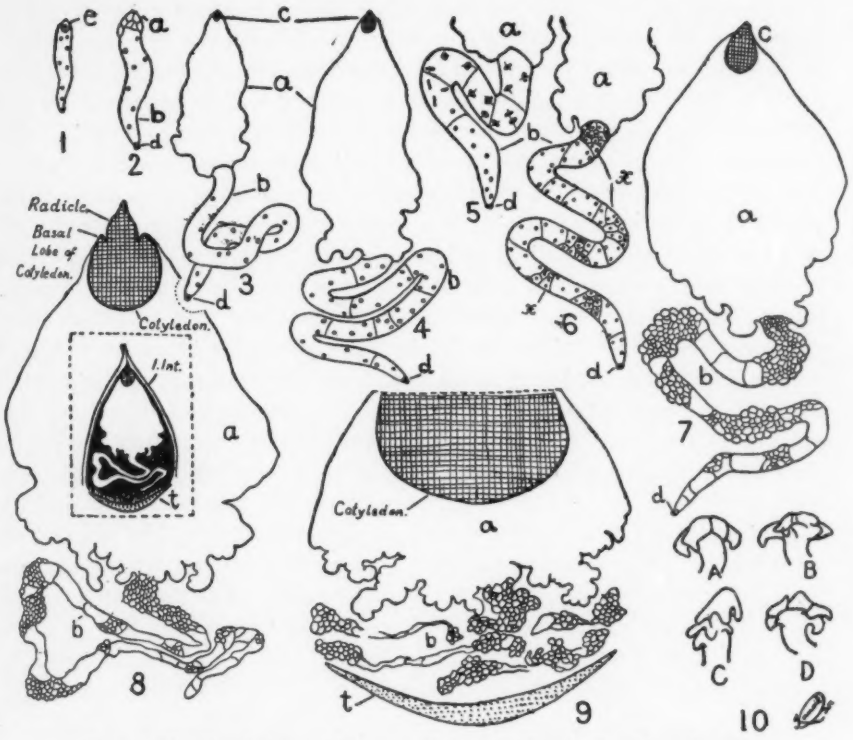
series of developmental stages in the seeds of *Grevillea robusta*, revealed the following sequence of events:

After fertilization when some free endosperm nuclei are formed, the embryo-sac begins to assume a slightly zig-zag appearance (Fig. 1). This becomes more pronounced a little later as the lower end of the embryo-sac grows rapidly in length and presents a worm-like appearance to form the *vermiform appendage* containing free nuclei, while the upper end shows the formation of a few endosperm cells (Fig. 2). Gradually, as the formation of endosperm cells becomes more and more progressive in the upper part of the embryo-sac, the lower part, constituting the cœnocytic *appendage*, increases very much in length and becomes a slender tubular coiled structure which assumes a haustorial rôle and invades the surrounding nutritive cells formed by the activity of the meristematic tissue situated at the base of the seed (Kausik, 1938). At this stage the worm-like appearance of the *appendage* is very striking, and its total length may be about 3 to 5 mm. (Fig. 3).

In slightly older preparations cross partitions are seen here and there in the *appendage* which consequently becomes divided into a number of compartments containing free nuclei (Fig. 4). In the living preparations the cytoplasmic movement in the *appendage* is checked at these partitions.

Subsequently, the nuclei in the compartments undergo mitotic divisions (Fig. 5), and a process of cell-formation is next initiated in the compartments to form groups of small cells (Fig. 6). Some of the cells so formed may at first contain two nuclei (as at *x* in Fig. 6) but finally every cell becomes uninucleate. However, since cell-formation does not proceed uniformly throughout the *appendage*, but may be suppressed at any stage in some portions, some of the compartments still remain, either entirely or in part, in a multinucleate condition (Figs. 6–8).

In the cell-groups referred to above, some of the marginal cells bulge out at a slightly later stage and thus become very prominent (Fig. 7). At this stage the endosperm tissue formed in the upper portion of the embryo-sac is more massive than before. It continues to grow rapidly and very soon attains its maximum size, so that the *vermiform appendage* becomes caught between the base of this large endosperm mass and the pad of tannin-cells found at the chalazal region of the seed. Consequently, portions of the *appendage* in which cell-formation does not proceed far begin to collapse and shrivel up later (Fig. 8), and thus the groups of cells formed in the rest of the *appendage* become separated into a number of detached masses of cells which appear like loose clusters scattered in the chalazal region (Fig. 9). By this



FIGS. 1–10. Development of the *vermiform appendage* in *Grevillea robusta*.

a, endosperm tissue; *b*, *vermiform appendage*; *c*, embryo; *d*, persisting antipodal cells: *e*, fertilized egg; *I.Int.*, inner integument; *t*, pad of tannin-cells at the chalazal region of the seed: the inset diagram in Fig. 8 shows the endosperm tissue and the *vermiform appendage in situ* in the seed. See text for explanations. All the figures are diagrammatic.

time the haustorial activity of the *appendage* is over, and the clusters of cells lying free in the space formed by the disorganization of all the nucellar cells can be easily scraped out by means of a needle. As the seeds grow older, even these clusters are completely used up, along with whatever remains of the rest of the endosperm tissue, by the rapidly advancing embryo, and the mature seeds thus become non-endospermic.

In some of the preparations there were seen some 3 to 4 or more large "horn-like" cells (Kausik, 1938) at the upper end of the *appendage* at the stage represented in Fig. 3. While they are not constantly met with in all cases, they appear to be very conspicuous when present, especially in stained preparations, and assume a variety of forms (Fig. 10, A to D).

In conclusion, it must be stated here that for studying the *vermiform appendage* the whole mounts of endosperm are found to be very satisfactory, in fact they are much superior to microtome sections, and the following are some of the advantages of the dissection method employed in making such preparations. The method involves only a little time and labour, so that a number of preparations may be made and examined in a surprisingly short time ; shrinkage and other distortions which invariably occur in materials prepared for sectioning with the microtome are eliminated in the case of entire preparations, but a very slight shrinkage, which, however, does not seriously interfere with observations, may sometimes be inevitable if the entire preparations have to be stained and mounted permanently; and lastly, the whole mounts prepared according to the dissection method seldom fail to give a correct and complete picture of the *appendage* provided a careful selection is made of the seeds at different stages. In the microtome sections one very obvious defect is that the coiled *appendage* does not come all in one section even when this is very thick, and it is not always practicable to cut unusually thick sections in the expectation of finding the endosperm tissue and the *appendage*, completely or almost so and in proper relations, in one and the same section. For these reasons, and since it must also be admitted that one has frequently to employ the technique which suits most the material to be studied, the importance accorded in the present instance to the dissection method is fully justified. Buchholz (1938) advocates a similar method for studying the tortuous suspensor systems in the case of the conifer embryos.

I wish to express my gratitude to Prof. B. Sahni, University of Lucknow, and Dr. P. Maheshwari, Dacca University, for their many kind courtesies. Further, I am sincerely thankful to Prof. M. A. Sampathkumaran for the encouragement and helpful criticism given to me at various stages during the course of this study.

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THIAMIN AND GROWTH OF SOME SPECIES OF *PYTHIUM*

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Introduction

It is a well-known fact that fungi can be classified into two main groups on the relation of thiamin to their growth. Group I includes those which require for growth an external supply of thiamin or its intermediates, while in Group II are included those which are capable of synthesising thiamin or its intermediates from the ingredients of nutrient media and do not require these from an extraneous source.

In a previous note[6] the author communicated that *Pythium hyphalosticton* Sideris probably belonged to Group II, and that the details of experiments, which needed verification, would be published in a subsequent paper.

The present investigation deals with the experiments carried out to come to definite conclusions. For the sake of comparison *Pythium arrhenomanes* Drechsler, *P. deliense* Meurs, *P. graminicolum* Subramaniam, and *P. mamillatum* Meurs were also used.

Methods

The following nutrient media were used:—

Medium A contained 0·5 gm. each of K_2HPO_4, $MgCl_2 \cdot 6\,H_2O$ and K_2SO_4, 2·0 gm. of NH_4NO_3, 5·0 gm. of pure dextrose and 1000 c.c. of distilled water.

Medium B contained all the ingredients of medium A, but in place of 2·0 gm. of NH_4NO_3 there were 1·0 gm. of purified asparagine and 1·0 gm. of NH_4NO_3.

Medium B *a* contained all the ingredients of medium B, but distilled water was only 250 c.c.

Medium C was medium C used by Robbins and Kavanagh.[5] It was composed of 5·0 gm. of $MgSO_4 \cdot 7\,H_2O$, 15·0 gm. of KH_2PO_4,

5·0 gm. of asparagine, 0·5 gm. of NH_4NO_3, 50·0 gm. of dextrose and 1 c.c. of mineral supplements per litre of distilled water. Its pH was 4·3.

Medium D was prepared by diluting medium C five times (*i.e.*, 100 c.c. of solution C + 400 c.c. of distilled water). Its pH was 4·5.

Medium E was prepared by diluting medium C ten times. Its pH was 4·75.

Medium F was prepared by adding sufficient quantity of KOH to medium C to make its reaction pH 5·3.

Medium G was prepared by diluting medium F five times. Its pH was 5·5.

Medium H was prepared by diluting medium F ten times. Its pH was 5·6.

Medium I was medium C to which was added thiamin (1 unit per c.c. of the solution*).

Medium J was medium F to which was added thiamin (1 unit per c.c. of the solution).

The methods and technique in general employed in this investigation were the same as described in previous papers.[6, 7, 8] Asparagine was purified by repeated precipitations with alcohol. Guaranteed reagents and thiamin (vitamin B_1) of Merck and Co. were used. A solution of thiamin in distilled water was prepared so as to give 25 international units in 1 c.c. of the solution, which was added to some media before autoclaving. The pure dextrose was dextrosol from the Corn Products Co.

For sterile filtration Seitz sterile filter was used. For solidifying the media Difco bacto-agar was employed.

The species of *Pythium* and the test fungi used (*i.e.*, *Phytophthora erythroseptica* Pethybridge, +strain of *Phycomyces blakesleeanus* Burgeff, *Phytophthora fagopyri* Takimoto and *Mucor ramannianus* Moller.) were maintained on potato dextrose agar and oatmeal agar. A bit of mycelium from the stock cultures was used as inoculum in each case. Care was taken in inoculating the solutions to avoid transferring any of the agar medium or touching the agar with the transfer needle. All cultures were grown in triplicate. The range of temperature during the experiments was 25–27° C.

* 1 unit is 10^{-9} Mole of the compound.

The quantity of the media used was 25 c.c. in each 150 c.c. Erlenmeyer pyrex flask and 50 c.c. in each Petri-dish (14 cm. $\times$2 cm.).

Dry weights were determined by washing the mycelium thoroughly with distilled water and drying at 60° C. for 72 hours.

Experimental

Media A, B, C and F were inoculated with the test fungi, but there was no appreciable growth in any case indicating that the media were free from the significant amount of thiamin or its intermediates. The test fungi responded if thiamin was added to these media.

The following experiments were performed:—

Series I.—Flasks containing medium A were inoculated with the five species of *Pythium*. It was found that they grew well on this medium and were indefinitely transferable on it.

Series II.—60 c.c. of medium A was poured in each of the flasks which were autoclaved and were inoculated with the species of *Pythium*. The flasks were then kept in diffused light for 14 days. It was noticed that the colonies of the fungi ramified in the medium and also formed mycelial mats on its surface.

(*a*) In the case of each fungus the mycelium of the three flasks was removed, washed well in distilled water, which was changed several times. It was ground and added to 200 c.c. of medium B, which was then heated in an autoclave for 5 minutes at 5 pounds pressure, filtered sterile and poured in five sterilized flasks. These were then incubated for 3 days at 30° C. The uncontaminated ones were inoculated with the test fungi (Plate IX, Fig. 1).

(*b*) 25 c.c. of medium B *a* was added in the case of each fungus to 125 c.c. of the medium from which the mycelium was removed as described under Series II *a*. The pH of the medium was adjusted to 7 by N/10 KOH solution and it was then filtered sterile, poured in five sterilized flasks. The flasks were then incubated for 3 days at 30° C. The uncontaminated ones were inoculated with the test fungi.

It was found that the test fungi gave appreciable growth in all the experiments in the case of each fungus. *Phycomyces blakesleeanus* comparatively formed more sporangia when it was grown on medium B containing the extracts of the fungus colonies.

In some cases aerial growth was not sufficient to allow the taking off suitable inocula with ease. Therefore, all the experiments already described

were repeated and in each case a small inoculum was cut off from the margin of a young colony of each fungus growing on medium A with Difco bacto-agar, on which it was transferred from potato dextrose agar or oat-meal agar. The results obtained were the same as already described.

There is no doubt that Difco bacto-agar contains biotin and traces of thiamin or its intermediates as has been reported by Robbins[3] but the experience of the author is that the amount of growth substances contained in a bit of Difco bacto-agar carried along with an inoculum is immaterial for such experiments. Leonian and Lilly[1] have also used bacto-agar with success for their experiments demonstrating the effect of thiamin on the growth of many fungi.

Series III.—Medium B was supplemented with thiamin and experiments were performed in Petri-dishes and flasks. Medium B was used as control. In the case of each fungus the inoculum (4 mm. in diameter) was cut off from the margin of the young colony growing on solidified medium B.

TABLE I

Diametric spread in cm. of the fungus colonies on solidified medium B

Fungi	At the end of	Without thiamin	Supplemented with thiamin, each Petri-dish containing	
			10 units	50 units
	hrs.			
P. arrhenomanes	24	3·0	3·1	3·3
	48	6·3	6·2	6·6
P. deliense	24	6·1	6·1	6·0
	48	11·8	11·9	11·9
P. graminicolum	24	4·4	4·4	4·4
	48	8·6	8·1	8·4
P. hyphalosticton	24	3·7	3·7	3·5
	48	7·3	7·4	7·6
P. mamillatum	24	3·8	3·9	3·9
	48	6·7	6·7	6·7

Growth of each fungus on the medium with or without thiamin was nearly of the same type so far as macroscopic examination indicated.

TABLE II

Dry weight of mycelium (in milligrams) of the fungi grown for 10 *days in flasks on medium B, and on medium B supplemented with thiamin*

Fungi	Medium B	Medium B +5 units	Medium B +25 units
P. arrhenomanes	37	36	40
P. deliense	35	32	37
P. graminicolum	27	30	28
P. hyphalosticton	29	28	30
P. mamillatum	26	24	24

Series IV.—It is reported by Robbins and Kavanagh[3] that *P. arrhenomanes* and *P. hyphalosticton* do not grow on their concentrated medium C; only the former responds when vitamin B_1 is added to the medium. Experiments were, therefore, performed to determine the effect of the concentration of media on their growth.

Flasks containing media C, D, E, F, G, H, I and J were inoculated with *P. hyphalosticton* and *P. arrhenomanes*. It was found that the former grew on all the media except C, F and I while the latter did not grow on media C and F only.

Discussion and Conclusions

Since the five species of *Pythium* grew well and were indefinitely transferable on medium A which was free from the significant amount of thiamin or its intermediates, it is clear that the fungi do not require organic growth supplement from an external source for their growth.

It is a well-known fact that the test fungi used require an external supply of a growth supplement[1,2] and the author has demonstrated that they do not grow on medium B. Since they grew well on medium B when the extracts of mycelium of the species of *Pythium* were added to it [*vide* Series II (*a*)], it can safely be assumed that the required growth-promoting substance was present in the extracts and that it was contained inside the mycelium of the *Pythiums*, which must have synthesised it from the ingredients of medium A on which they were allowed to grow. Similarly from the results of experiments of Series II (*b*) it is evident that some of the synthesised growth substance was also given off by the mycelium of the *Pythiums* into medium A.

The synthesised growth substance was probably thiamin, but this cannot be assumed with certainty since it has not been demonstrated as yet that *Phytophthora erythroseptica* responds to thiamin only and to no other growth substance. It is not also possible to say whether pyrimidine or thiazole or both formed a part of the synthesised growth substance since the other three test fungi used respond to thiamin also. In short, the exact nature of the growth-promoting substance cannot be stated.

These species of *Pythium* behave like *Cunninghamella blakesleeana* Lender (**1**, p. 540) and *Saprolegnia delica* Coker[8] which are also capable of synthesising their growth-promoting substance from the ingredients of nutrient media.

From the results of experiments of Series III summarised in Tables I and II it is clear that the addition of thiamin has no marked effect on the growth of the fungi (see also Plate IX, Fig. 2). In this respect they resemble *Agaricus campestris* Linn., *Absidia glauca* Hagem, *Basidiobolus ranarum* Eidam, *Cunninghamella* sp. no. 102, *Mucor circinelloides* v. Tiegham, etc.,[5] and *Saprolegnia delica*.[8] They differ from *P. aphanidermatum* (Eds.) Fitz. (*P. Butleri*), growth of which is augmented 70 times if the medium used by Robbins and Kavanagh (**4**, p. 454) is supplemented with thiamin. These results also indicate that the five species of *Pythium* synthesised the required quantity of growth-promoting substance and, therefore, an external dose of thiamin did not increase their growth.

Since *P. arrhenomanes* and *P. hyphalosticton* did not grow on concentrated media C and F but grew well when they were diluted five or ten times, *i.e.*, on media D, E, G and H (Plate IX, Figs. 3, 4), it is evident that the concentration of media C and F interfered with the growth of the two fungi.

The question naturally arises as to how the growth of *P. arrhenomanes* and *P. hyphalosticton* is interfered with in concentrated media C and F. Is it that the total concentration or concentration of sugar or some particular salt (*a*) does not allow the solutions to be assimilated, or (*b*) interferes with the synthesis of some necessary growth supplement in the solutions, or (*c*) interferes with the functioning of the growth supplement, if synthesised at all?

Had the first explanation been right there would have been no growth in solution J since the concentration of solutions F and J was the same.

The assumption that the concentration interfered with the synthesis of some growth supplement rather than its functioning would seem to follow

since the addition of the vitamin to solution F made the organisms grow (*i.e.*, in solution J); it was effective if present. This assumption is further supported by the fact that the fungi can grow well in solutions C and F when they are diluted, *i.e.*, on media D, E, G and H. Since they grow well on these media, which are free from thiamin or its intermediates and since they require no external supply of growth supplement, it can safely be assumed that they must be synthesising their growth substance from the ingredients of media D, E, G and H. It is, therefore, clear that in diluted media the synthesis of the growth supplement is not interfered with.

The other result to be interpreted is the failure of *P. hyphalosticton* to grow in solution I (Plate IX, Fig. 3, I), the concentration and composition of which is the same as that of solution J; the only difference is that the former does not contain KOH and, therefore, it is more acidic than the latter. It may be that the lower pH or the concentration of solution I makes the added vitamin ineffective, but this explanation cannot be true since the fungus is capable of growing and synthesising growth-promoting substances on medium D with a pH 4·5 and it grows on medium J, the concentration of which is practically the same as that of I. The author is inclined to think that *the lower pH in concentrated solution I* makes the vitamin ineffective when it is added.

Summary

1. The nutrient solution used containing mineral salts and pure dextrose does not contain any appreciable amount of growth-promoting substances.

2. *Pythium arrhenomanes*, *P. deliense*, *P. graminicolum*, *P. hyphalosticton* and *P. mamillatum* are capable of unlimited growth in the nutrient solution.

3. The results of the experiments show that the organisms are capable of synthesising their own growth-promoting substance from the elementary ingredients of the nutrient solution.

4. Some of the growth-promoting substance manufactured by the organisms is also given off by the mycelium into the solution.

5. The addition of thiamin to nutrient medium B has no marked effect on the growth of these organisms.

6. The failure of *Pythium arrhenomanes* and *P. hyphalosticton* to grow in the synthetic liquid medium used by Robbins and Kavanagh is found to be due to the concentration of the medium. It is suggested that the

concentration hinders the synthesis of growth-promoting substance and thus affects the growth.

The author thanks his colleague, Mr. A. K. Mitra, M.Sc., for taking the photographs reproduced in this paper.

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EXPLANATION OF FIGURES

Plate IX

Fig. 1.—Growth of test fungi (B = *Phycomyces blakesleeanus*, E = *Phytophthora erythroseptica*) in the mycelial extract of *Pythium deliense*.

Fig. 2.—Growth of *P. arrhenomanes* on medium B supplemented with thiamin. O = control, *i.e.*, medium B without thiamin, 5 = medium B + 5 units of thiamin, 25 = medium B + 25 units of thiamin. Its growth seems to be unaffected by the addition of thiamin so far as macroscopic examination indicates. (For dry weight of its mycelium please see Table II.)

Fig. 3.—Growth of *P. hyphalosticton* on concentrated and diluted media. Little or no growth on concentrated media C and I (I is medium C + thiamin) but good growth on diluted medium D (D = medium C diluted 5 times).

Fig. 4.—Growth of *P. arrhenomanes* on concentrated and diluted media. No growth on medium C but good growth on media D and I.

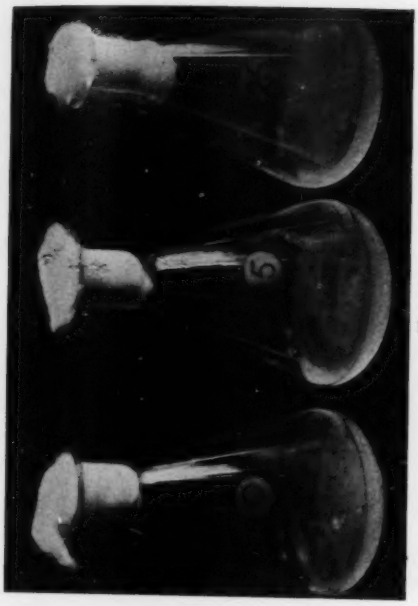

FIG. 2

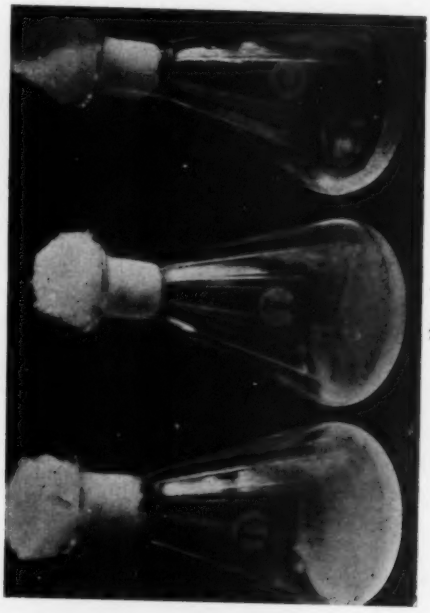

FIG. 4

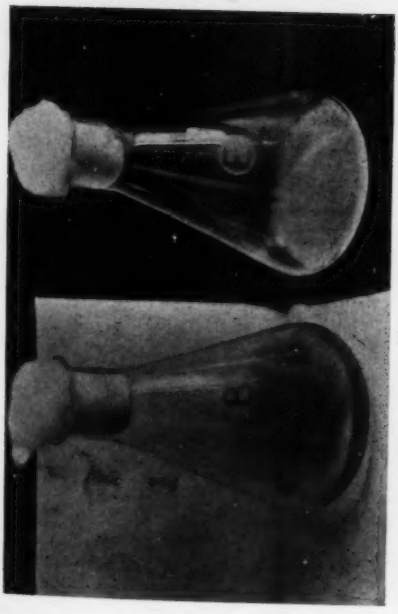

FIG. 1

FIG. 3

*CYTO-MORPHOLOGICAL STUDIES IN *ASTERACANTHA LONGIFOLIA* NEES. *(HYGROPHILA SPINOSA.* T. AND.)

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I. Introduction

The name of this family, Acanthaceæ to which *Asteracantha longifolia* Nees. belongs, comes from the typical genus, *Acanthus*. *Acanthus* is a Greek term (Acanthos) meaning a spine (Bailey, 1933). Since several species of this genus *Acanthus* (*Acanthus ilicifolius*) and other genera (*Barleria*, *Asteracantha*) under this natural order possessed spines or thorns, the family came to be called Acanthaceæ or more popularly *Acanthus* family. The members of this family confined mostly to the tropical and sub-tropical regions of the world, present certain very interesting morphological and ecological characters. Cytologically, they seem to be no less important. However, work both cytological and morphological in this family of diverse habit has been but

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scanty. While other families like Scrophulariaceæ, Gesneneriaceæ, Pedaliaceæ, Labiateæ, Verbenaceæ belonging to the Tubifloræ (Rendle, 1938), or the Bi-carpellateæ (Benthm and Hooker, 1682–83), have been sufficiently dealt with, literature on Acanthaceæ is very small. Schnarf (1931) speaks of a number of authors (Hartmann, Gigante) who have given a short account of the development of the mega and the microspores and the nature of the endosperm. Schürhoff (1926) has similarly given a brief catalogue of the various kinds of haustoria in a few members that were noted by different authors, during embryogeny. Karsten (1891) studied the embryo-sac of the genus *Acanthus*, but was uncertain about some details. Maheshwari (1937) has recommended reinvestigation of this. Only meagre information is available about the following genera:—*Acanthus* (Gigante, 1929); *Strobilanthes*, *Ruellia*, *Eranthemum*, *Barleria*, *Acanthus*, *Crossandra*, *Aphelandra*, *Cryptophragmium*, *Schaueria*, *Belloporone* (Hartmann, 1923); *Acanthus spinosus* (Hofmeister, 1858–59); *Thunbergia* (Juel, 1915; Strasburgher, 1882); *Acanthus* (Tieghem, 1908) and *Eranthemum* (Vesque, 1878).

Karyological studies in this family have been very few. The meiotic chromosomes of the members of this family were not known until Gigante counted them in *Acanthus mollis* in 1929 though even that count was quite uncertain.

Thus it would appear that though some members of this family have been the objects of investigation in the past at the hands of various authors, *Asteracantha longifolia* Nees., a tropical plant of the Indian soil which is supposed to be a sudorific and a somewhat bitter tonic, though very troublesome because of the sharp thorns, is interesting in more ways than one. In the present paper, the development of the embryo-sac and pollen, growth of the embryo and endosperm, and some details relating to the anatomy of the stem, root and leaf, have been described. Besides, the morphology of the conspicuous thorns and the floral tip has also been studied. The meiotic and the somatic chromosome numbers have been determined for the first time.

II. Material and Methods

Asteracantha plants were available in plenty in the neighbourhood of pools, ponds, streams and ditches of Annamalainagar. Especially during the rainy season when they are most exuberant, it was easy to get good root-tips from the swampy marshes by gentle pulling. Root-tips were fixed at about 10 A.M., when the formative cells were in a state of active division, in chrom-acetic formalin of Karpechenko and Langlet (Manton, 1932) without prefixation in carnoy. Anthers of the desired stages of development were

first determined by the acetocarmine smear method and then fixed in chrom-acetic formalin. For ovaries and ovules of different stages of development, either formalin-acetic-alcohol or hot corrosive sublimate was used. In order to study the organogeny of the flower, floral tips were fixed in corrosive sublimate, which gave good results. Mature seeds were first soaked in glacial acetic acid for a few days before fixation in order to soften the tissue and facilitate sectioning. Materials were dehydrated in alcohol, cleared in chloroform and imbedded in paraffin. Sections were cut at thicknesses varying from 5–20 microns. For sections of anthers and root-tips both Heidenhain's iron-alum hæmatoxylin and Newton's iodine-gentian violet were used as the stains, while for the preparations of ovaries, ovules, seeds and floral tips, hæmatoxylin was exclusively employed.

III. Description of the Plant

This is a stout, erect herb 2–5 ft. in height growing abundantly in marshy places on the brinks of streams, pools and ditches. They are profusely common particularly near paddy fields. Branching is sparse and the plant body is covered by numerous hispid hairs. Outwardly spreading stiff woody thorns, six in number at each node, form the most conspicuous part of the plant. The nodes are swollen like those of its congeners in the family and the stem is somewhat pink in colour.

Leaves, opposite and decussate, linear-lanceolate, subsessile with acuminate tip and hairy.

The axillary buds in the axils of the two opposite leaves become more often transformed into thorns than develop into branches. Each of these two thorns bears at its base one on either side, two more leaves shorter than the first two. So, for the two thorns there are four smaller leaves and these four again bear in their axils more thorns. At the base of each one of these four thorns two more leaves arise and the buds in the axils of the latter develop into flowers. Thus there are actually three whorls of leaves arising one above the other as do the floral leaves in a flower, the lowermost whorl consisting of the two big opposite leaves. This seems to confirm the view already expressed by Raghavan and Venkatasubban (1941) supporting the classical concept relating to the morphology of the flower. From the axil of each of these two big leaves on the main stem, therefore, there appear three thorns, six leaves, and four flowers. Since the uppermost or the innermost whorl of small leaves bears immediately in their axils flower buds, these leaves can be regarded as the bracts. All these parts are sessile and are crowded at the base and form a cluster.

The blue-purple sessile flower has a pair of membraneous linear bracteoles. Each of these bracteoles sometimes bears in its axil another flower bud and this has its own bracteoles and so on. Calyx, deeply 4-partite; corolla of five petals, bilabiate ; upper lip of two petals, the lower of three, with two crested folds on the palate. Stamens, 4, didynamous, anthers two-celled, the lobes standing parallel and at the same level. Ovary, bicarpellary, syncarpous with axile placentation; it is surrounded at the base by a prominent fleshy annular nectariferous disc; ovules in the young stages are arranged obliquely in the carpels with their chalazal ends pointing upwards. Style filiform. Stigma is bilobed in very early stages, later becoming simple by the suppression of one of the lobes. Fruit is a linear oblong loculicidal capsule covered by white hairs, with hook-like funicular projections, the jaculators, (Figs. 47, 48, 49 and 50) which help in their dispersal.

IV. Anatomy of the Stem, Root, Leaf and Thorn

The stem shows a herbaceous type of vascular system (in which the vascular tissue is in the form of discrete bundles, big and small, arranged in a square or rectangle) undergoing secondary thickening. The pith and cortex are composed of very loosely arranged parenchymatous cells with large intercellular spaces. Solereder (1908) refers to this as the lacunar cortex. Conspicuous collenchyma forms a broad hypodermal zone. Calcium oxalate crystals, in the form of very long isolated needles, are distributed throughout the stem. Besides, cystoliths though more common in leaves, are also found in the stem. The epidermal hispid hairs are multi-cellular and their cytoplasm is almost closely packed with calcium oxalate needles which are comparatively much smaller than those of the stem. The pink colour of the stem is due to anthocyanin pigment in some of the epidermal cells.

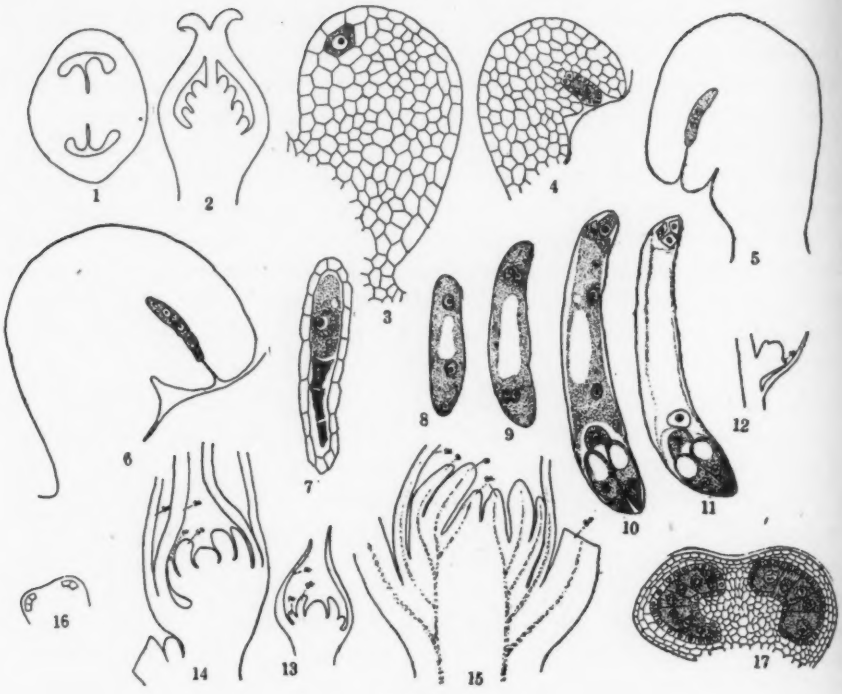
The cortex is filled with large air-chambers separated by narrow bridges of cells that connect the hypodermis and the cells that border upon the endodermis. There is no well-defined epidermis, but like the monocot root, possesses an exodermis with a thick outer wall scarcely distinguishable from the inner cortical cells. The endodermis is seen clearly with the conspicuous Casparian bands. It is interesting to note in this connection that the plant is capable of vegetative propagation especially during favourable conditions, because of adventitious roots springing from some nodes above the soil. These roots form stilts just as in *Pennisetum* or *Andropogon*. While that part of the adventitious root below the soil shows the normal root structure the part above the soil shows anatomical features that are intermediate between stem and root. It has a fairly large pith, but it has also very large air-chambers. In its habit, *Asteracantha* is partly aquatic and so it has got a well-developed aerating system.

The leaves of some members of the family have been described as centric and some as bifacial (Solereder, 1908). The leaf of *Asteracantha* is not bifacial, but centric, *i.e.*, having palisade tissues towards both upper and the lower surfaces. There is no hypodermis and the stomata which appear in a surface view to be capped by a subsidiary cell, were found to occur on both sides. Cystoliths abound in the epidermal cells as elongated club-shaped bodies blunt at both ends. Solereder speaks of the attempt of a certain author named Hobein who has classified Acanthaceæ into various tribes according to the shape, size and position of the cystoliths. Hobein places *Asteracantha* in the tribe, Ruellieæ, which is characterised by cystoliths of varying shapes. The chromosome number of the plant also seems to support this.

The morphology of the thorn has not yet been investigated critically. Three important factors go to support their axillary origin. The most familiar fact is that they arise in the axils of the leaves. Secondly their internal structure especially in the younger stages of development, is an exact prototype of that of the stem or branch, though the thorn becomes soon hardened into a stiff structure by every thick-walled sclerenchyma. This almost fills the inner part, especially the vascular region even to the exclusion of the vessels (Fig. 52). Thirdly these thorns themselves in luxurious plants were seen to bear on them nodes and internodes, small caducous leaves and tiny axillary branches which also sooner or later become transformed into stiff branch thorns.

V. Organogeny of the Flower

The primordium of the flower first makes its appearance as a knob-like protuberance in the axil of a leaf (Fig. 12). The order of development of the floral whorls was found to be calyx, androœcium, corolla and gynœcium. Schertz (1919) noticed the same order of formation of the floral whorls in *Scrophularia marylandica*. Similarly Srinivasan (1940) has observed the same in *Angelonia grandiflora*. Both these plants belong to Scrophulariaceæ, a family closely allied to Acanthaceæ in the Tubifloreæ. The primordia of the sepals arise as lateral lobes from the apical dome (Fig. 12 *se*). These enlarge and are soon accompanied by the appearance of the primordia of the stamens and not by those of the petals. The appearance of these is much like that of the sepals. By this time the sepals have very much elongated because of their rapid growth and are many times longer than the stamens (Fig. 13). By now the primordia of the petals have already become initiated at a slightly lower level than the stamens. They soon overtake the stamens in their growth (Figs. 13 and 14). Lastly the two carpels arise from the dome-shaped apex. Careful observation shows that these two carpels are not



FIGS. 1–17

FIG. 1. T. S. of ovary. × 150. FIG. 2. L. S. of ovary. × 75. FIG. 3. Hypodermal archesporium in the ovule. × 750. FIG. 4. Megaspore-mother cell surrounded by a single layer of nucellus. × 355. FIG. 5. Dyad. × 355. FIG. 6. Linear tetrad. × 355. FIG. 7. Uni-nucleate embryo-sac. × 1500. FIG. 8. Bi-nucleate embryo-sac. × 750. FIG. 9. 4-Nucleate embryo-sac. × 750. FIG. 10. 8-Nucleate embryo-sac. × 750. FIG. 11. 7-Nucleate embryo-sac. × 750. FIG. 12. Flower primordium showing the origin of sepal at *se.* × 150. FIG. 13. Shows the origin of the stamens (*st.*) and petals (*pe.*). × 150. FIG. 14. Do. a later stage. × 150. FIG. 15. Lateral origin of carpels, showing unused apex. × 75. FIG. 16. Archesporial cells of the anther. × 75. FIG. 17. The anther wall is composed of four layers of cells. The inner-most of which is the tapetum and is uni-nucleate. × 355.

exactly terminal in origin on the central dome, but are lateral to it (Fig. 15). The lateral nature of the carpel origin has been employed by Newman (1936), Raghavan (1937) and Srinivasan (1940) in confirming the classical foliar concept of the carpels.

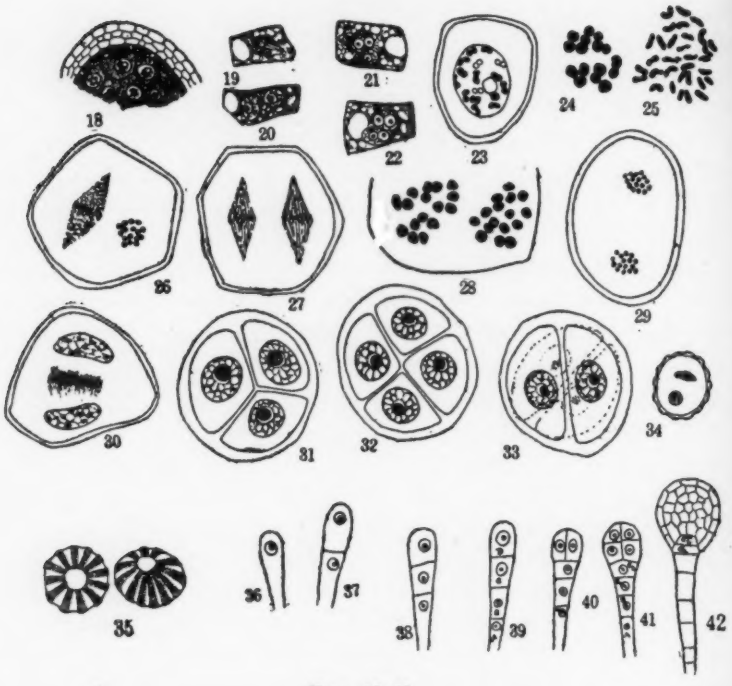
VI. Development of the Microspore

All the four anthers in the flower are fertile. The pollen-mother cells are comparatively larger while the chromosomes are small. At the time when the anther appears four-lobed in the cross-section, the primary archesporium

of the microsporangium just makes its appearance as three or four cells in a hypodermal fashion (Fig. 16). So the archesporium is not very extensive. These archesporial cells of the anther can easily be recognised amidst other cells of the anther by their larger size, bigger nuclei and denser cytoplasm. The lobes of the anthers become more and more conspicuous after the initiation of the archesporium. The archesporial cells divide periclinally giving rise to a layer of primary wall cells or parietal cells on the outside and a layer of primary sporogenous cells on the inside. The primary wall cells repeatedly divide periclinally forming three layers of wall cells of which the innermost functions as the tapetum (Fig. 17). The layer of wall cells immediately outside the tapetum get tangentially elongated.

The tapetal cells enlarge and in the beginning, each has a single conspicuous nucleus and deeply staining cytoplasm. This nucleus undergoes mitotic division just when the prophase of the pollen-mother cell nuclei is well under way (Fig. 18). Bi-nucleate tapetal cells were commonly seen while a solitary case of a tri-nucleate tapetal cell was also met with (Fig. 20). Some authors have mentioned amitosis or fragmentation in regard to the division of the tapetal cell nucleus. Suguira (1936) has spoken of the likelihood of such a division, while O'Neil (1920) found that the nuclei in the tapetal cells of *Datura stramonium* divide by amitosis. But Bonnet (1912) and later Cooper (1933) have found in several cases in the Angiosperms that the tapetal nuclei divide by mitosis. The latter has grouped 43 species of Angiosperms in a critical study into three classes according to the behaviour of the tapetal nucleus: (1) in which the tapetal cell throughout remains in a uni-nucleate condition without division ; (2) in which the nucleus divides once and remains bi-nucleate ; and (3) in which multi-nucleate tapetal cells are present. Large vacuoles form a conspicuous feature of the tapetal (Figs. 20–22) cells though Joshi (1936) has reported the complete absence of the vacuoles in the tapetum of *Stellaria media*. The tapetum in the latter stages of development of the pollen mother cells disorganises and disintegrates. The pollen mother cells enlarge and remain closely packed in the early stages. But at about the stage of diakinesis they begin to round off.

Diakinesis.—In this stage sixteen pairs of homologous chromosomes are distributed in the periphery of the mother cell in a very condensed form. The nucleolus itself appears to be placed in a slightly peripheral position. The bivalents are approximately placed in a equidistant manner. This equidistant disposition of the bivalents according to Lawrence is due to a repulsion phase which commences at early diakinesis and continues till mid-diakinesis and this suggests that the inter-bivalent repulsion is almost equal.



FIGS. 18–42

FIG. 18. Bi-nucleate tapetum. × 350. FIG. 19. A tapetal cell showing the mitotic division of its nucleus. × 1500. FIGS. 20–22. Tapetal cells showing various stages of nuclear fusion. × 1500. FIG. 23. Diakinesis. × 1500. FIG. 24. Metaphase I showing secondary association. × 3900. FIG. 25. Somatic metaphase; $2n = 32$. × 3900. FIGS. 26 and 27. Two views of Metaphase II. × 1500. FIGS. 28 and 29. Metaphase II. Fig. 28 shows the persistence of secondary association. × 3900. FIG. 29. × 1500. FIG. 30. Dyad. × 1500. FIGS. 31–33. Three types of pollen tetrads. × 1100. FIG. 34. Bi-nucleate pollen grain at the time of shedding. × 355. Note the lenticular shape of the generative cell. FIG. 35. Two views of the mature pollen grain. × 350. FIGS. 36–42. Various stages of the development of the embryo. × 750.

Then the pairs of chromosomes become very much contracted so that their bivalent nature can hardly be recognised. What appeared to be ring-bivalents were noted in this plant (Fig. 23). But it is not quite certain.

Metaphase.—The contracted bi-valents sixteen in number then arrange themselves in the equatorial plane (Fig. 24). Secondary association seems to be prevalent in this plant, but sufficient number of metaphase plates were not available to illustrate the different kinds of secondary association. Catcheside (1937) found that the bi-valents in secondary association in the first metaphase are those that have occupied adjacent positions in the diakinesis. In the

metaphase that I obtained, the number of secondary associations is eight. Out of the sixteen bi-valents three are free while remaining thirteen have entered into association. There are three secondarily associated groups of three bi-valents each and two such groups of two bi-valents each. In the present case secondary association was seen to persist even in the second metaphase (Fig. 28). Catcheside, however, reports that in *Brassica* secondary association disappears during anaphase I. The haploid number was confirmed by somatic count (Fig. 25).

Then, after the segregation of the chromosomes for which Kuwada (1929) assumes that polar attraction is responsible, they reach the poles and reorganise themselves into inter-kinesis nuclei. The nucleolus appears and the chromosomes are uniformly placed, connected by strands (Fig. 30). This uniform spacing of chromosomes at inter-kinesis was first recorded by Gates (1909) in *Oenothera* and again by Raghavan (1938) in *Gynandropsis*. Cross-wall seemed to be formed by cell plate. But it was hazily seen. The result is the formation of a dyad. The two daughter nuclei then divide simultaneously (Figs. 26 and 27). Fig. 29 shows two groups of sixteen chromosomes each in second metaphase polar view. Secondary association is to be seen upto this phase (Fig. 28).

In the second metaphase the spindles of the two groups of sixteen univalents may be placed in the same plane (Fig. 27) both showing side view or the polar view (Fig. 29). Or they may be placed in two planes which are perpendicular to each other (Fig. 26). Now, when the tetrads are developed, their relative dispositions depend upon the spindle positions in the II metaphase. The two spindles showing the side view (Fig. 27) develop into a tetrad of four microspores or pollen grains which are all to be seen in one focus (isobilateral) (Fig. 32), while the spindles placed at right angles to each other in the same phase develop into a tetrad wherein three spores are seen in one focus and one in another. In other words, this is the tetrahedral arrangement of spores (Fig. 31). In the other polar view of the former case two spores only are to be seen in one focus, the other two in another (Fig. 33). Thus three types as it were of tetrads were noted (Figs. 31, 32 and 33). The mature pollen grain at the time of shedding is distinctly bi-nucleate (Fig. 34). Fig. 34 shows the lens-shaped generative nucleus. The diploid number of chromosomes was found to be 32 in the somatic cells of the root-tip (Fig. 25).

The pollen grains of the Acanthaceæ in general present wide variations in the sculpturing of their walls. Wodehouse (1935) deals with this in an elaborate manner, but no mention of Acanthaceæ pollen grains has been made. All these sculpturings are beautiful and in *Asteracantha*, particularly

they appear as thickening bands radiating from a central circular position on the wall (Fig. 35). So varied and at the same time so definite are these sculpturings on the pollen grains in the various plants of the Acanthaceæ, that Rendle speaks of a classification of this family into tribes according to the types of pollen grains.

VII. Megasporogenesis

The ovary is bi-carpellary typical of the bi-carpellate, of Benthem and Hooker. About four ovules arise as tiny protuberances in each carpel and are obliquely arranged with reference to the axile placenta. The archesporium of the megaspore arises as a hypodermal cell which is much bigger than the surrounding cells. Its cytoplasm is dense and nucleus large and prominent (Fig. 3). The archesporium is initiated earlier than the formation of the integument (Fig. 3). Such an early differentiation of the primary archesporium has been reported by Joshi and Rao (1934) in *Digera*, Langdon (1934) in *Carya* and *Juglans*, Woodroff (1928) in *Hicoria pecan* and Srivastava (1939) in *Orobanche ægyptiaca*. By this time the anthers show meiotic phases in the pollen mother cells. The flower of *Asteracantha* is therefore protandrous. This appears to be the rule not only in a majority of families, but also in almost all the members of the Acanthaceæ.

The archesporial cell enlarges and functions as the megaspore-mother cell (Fig. 4). The single integument characteristic of the Gamopetalæ develops enormously and the bulk of the ovule is more or less made up of the integument. The megaspore-mother cell is enveloped by a single layer of narrow cells which stain lightly compared to the other cells of the ovule. There is no other parietal tissue, the hypodermal cell directly developing into a megaspore-mother cell without cutting off any primary wall cell. This enveloping layer is regarded as the nucellus which, when the embryo-sac has been formed, disappears by disorganization. Such an early disorganization of the nucellus during the developmental stages of the embryo-sac appears to be a characteristic feature, of Acanthaceæ, Scrophulariaceæ (Srinivasan, 1940) and other closely related families. Balicka-Ivanowska (1899) calls this ' nucelle ' and has reported its prevalence in *Pedicularis palustris* (Scrophulariaceæ), *Klugia notoniana* (Gesnerriaceæ), *Campanula rotundula* (Campanulaceæ), etc., Bhaduri (1935) has a similar report about a number of solaneceous plants. In Lobeliaceæ too, the same was noted by Kausik (1938) in *Lobelia nicotianæfolia*, and by Srivastava (1939) in *Orobœncheæ*. Schertz (1919) is of the opinion that the nucellus in the micropylar region is destroyed by the elongation of the embryo-sac while towards the chalazal part it persists till its disorganization during endosperm development. In *Asteracantha*, however, it becomes destroyed even earlier.

No tapetum was observed in this. Mitchell (1915) reports its absence in *Striga lutea* ; Srinivasan (1940) in *Angelonia grandiflora.*

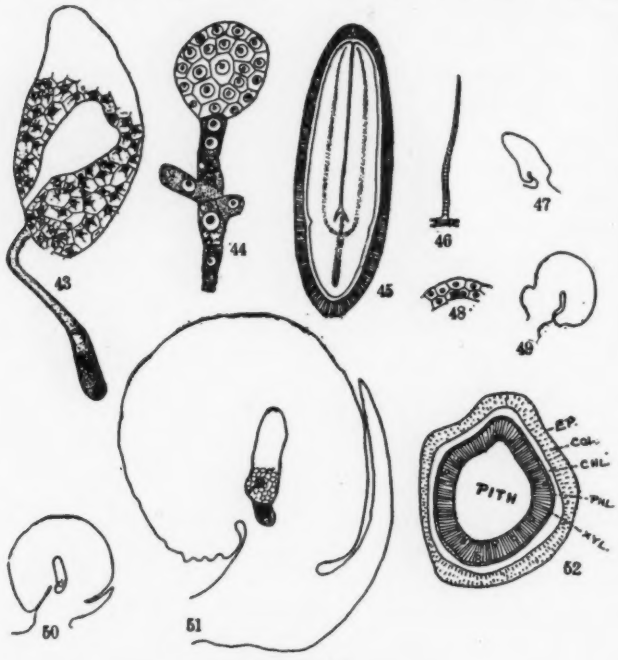
The megaspore-mother cell by a heterotypic division gives rise to a dyad (Fig. 5). The dyad by homotypic division forms a linear tetrad of megaspores, the cell nearest to the chalazal end developing into the embryo-sac (Fig. 6). This enlarges and vacuoles appear while the nucleus remains about the centre of the cell. This is the uni-nucleate embryo-sac (Fig. 7). This nucleus divides and forms a bi-nucleate embryo-sac with a vacuole in the middle and the nuclei at the ends (Fig. 8). This in its turn develops into a four-nucleate embryo-sac by further division (Fig. 9). The increase in the size of the embryo-sac almost comes to a standstill at this stage so that when subsequently the eight-nucleate embryo-sac (Fig. 10) is formed, there is not much difference between the former and the latter in size. Two nuclei, one from each end of the embryo-sac, migrate towards the centre and fuse so that at this stage the embryo-sac consists of only seven nuclei (Fig. 11). The meeting place of the polar nuclei is reported to be various by Schmid (1906) especially with reference to *Pedicularis palustris.* The embryo-sac is of the normal type (Maheswari, 1937). The mature eight-nucleate embryo-sac consists of two synergids, an egg cell, two polar nuclei and three antipodals. The egg-apparatus is prominent while the antipodals are not so. The embryo-sac appears to be curved owing to the crooked and faster growth of that integumental half farther away from the funicle.

Even slightly earlier than fertilisation the antipodals degenerate and are therefore of an ephemeral character. The synergids whose chief function is supposed to help in the fertilisation process having done its work disintegrate.

VIII. Embryo and Endosperm

The first division of the fertilised egg is transverse (Figs. 36 and 37). Further transverse divisions of the two cells thus formed produce a four-celled linear pro-embryo (Figs. 38 and 39). To facilitate description these four cells of the pro-embryo will be designated as A, B, C and D (Fig. 39). This stage of the embryo is an important one, as each one of them gives rise to a definite region in the mature embryo. A longitudinal wall laid down in the cell D produces a quadrant (Fig. 40). A transverse wall across the quadrant forms the octant (Fig. 41). The cells C_1 and C_2 (Fig. 41) are formed by the transverse division of the cell C. The cell C_2 is the hypophysis (Fig. 41). The suspensor is composed of three cells A, B and C_1. The embryo at the octant stage is six cells long. Fig. 42 represents a later stage in the development of the embryo. The primary meristems, dermatogen, periblem and plerome

have been differentiated. The hypophysis cell C_2 has undergone a transverse division to form two cells, C_2^1 and C_2^2 (Fig. 42). The upper of the two cells, (C_2^1) becomes continuous with the dermatogen while the lower C_2^2 forms part of the periblem (Fig. 42). In the mature seed the suspensor is about seven to eight cells long. This is due to the repeated division of the original three suspensor cells A, B and C_1. In one of the embryos two of the suspensor cells have put forth laterally haustorial protuberances



FIGS. 43–52

FIG. 43. Shows the bi-nucleate micropylar endosperm haustorium, the embryo and the cellular endosperm. × 150. FIG. 44. Suspensor haustoruim. × 700. FIG. 45. Mature seed in longitudinal section. × 75. FIG. 46. A hair of the seed coat. × 75. FIGS. 47–50. Various stages in the development of the jaculator. Fig. 48 shows the periclinal division of the hypodermal cells in the primordium of the jaculator. × 75. FIG. 51. Ovule showing micropylar haustorium, embryo and cellular endosperm and also the jaculator. × 75. FIG. 52. T. S. of thorn. *Ep.*, Epidermis; *Col.*, Collenchyma; *Chl.*, Chlorenchyma; *Phl.*, Phloem; *Xyl.*, Xylem. Note the scanty development of the vessels.

(Fig. 44). Though the haustorium observed was distinct, for want of sufficient data regarding this, nothing definite can be said about its origin, occurrence and function. Various kinds of haustoria, chalazal endosperm haustorium, micropylar endosperm haustorium and suspensor haustorium

have been recorded in the different genera of Acanthaceæ. Suspensor haustoria have been recorded in *Ruellia rosea* and *Eranthemum albo-maculatum* (Schürhoff, 1926). Lloyd (1902) in *Callipeltis cucullaria* (Rubiaceæ) has recorded such a suspensor haustorium (Schnarf, 1931). The mature seed consists of a more or less oblong embryo with rather long equal cotyledons. The cotyledonary part of the embryo is much longer than the hypocotyledonary region including the radicle. In the ripe seed the two plerome strands of the cotyledons meet those of the radicle at the hypocotyledonary region (Fig. 45). The plumule which is enclosed between the two cotyledons appears as a small papillate protuberance. The seed is non-endospermous. The cotyledons are replete with reserve food chiefly in the form of oil and starch. Fig. 45 shows not a fully mature seed so that a narrow portion of the endosperm is still to be seen. The testa is composed of two or three layers of cells. From this arise numerous very long multicellular hairs (Fig. 46), attenuating at their free ends developing when wet abundant mucilage which helps the seeds to stick to the soil during germination.

The dissemination of seeds is facilitated by hook-like structures called jaculators or retinacula. The first indication of the origin of the retinaculum is a knob-like protuberance from the funicle of the ovule and it grows with the ovule. Fig. 48 shows one of the hypodermal cells in division in the knob-like region. Some of the stages in the development of the jaculator are shown in Figs. 48 to 50. Fig. 51 shows a fully developed jaculator.

Endosperm.—The endosperm is cellular in development. Such a cellular endosperm seems to be a common feature in Sympetalæ. Cellular endosperm has been recorded previously by various authors. Hartmann (1923) has recorded the presence of cellular endosperm in *Acanthus longifolius*, while Gigante (1929) in *A. mollis* found the nuclear development of the endosperm. An endosperm haustorium was observed in the micropylar region of the ovule. The endosperm haustorium is a narrow irregularly curved tube-like structure. It is bi-nucleate (Fig. 43). It arises as a protuberance from a micropylar endosperm cell (Fig. 51) and grows towards the micropylar end of the ovule eating its way through the micropylar region. Such a micropylar endosperm haustorium has been found to be present in *Cryptophragmium ceylanicum* and *Acanthus spinosus* (Schürhoff, 1926). Chalazal endosperm haustoria have also been observed in *Acanthus longifolius*; *Crossandra undulæfolia* and *Barleria strigosa* (Schürhoff, 1926). The haustorium has a nutritive rôle namely supply of nutrition to the developing embryo, as has been determined in various other closely allied families, like Scrophulariaceæ, Gesneriaceæ, Lentibulariaceæ, Labiateæ, Verbenaceæ and many others besides.

IX. Discussion

The prevalence of haustoria in one form or other is a common feature in the several genera of the Acanthaceæ. Different types of endosperm haustoria, chalazal and micropylar, and suspensor haustorium have been reported to occur. In some genera one type of haustorium may be present while in others more than one may occur. In the present case two types, the micropylar endosperm haustorium and the suspensor haustorium, were noted.

Hobein (Solereder, 1908) has classified Acanthaceæ into different tribes on the basis of the presence or absence, shape and position of the cystoliths. In this classification, he has in the main followed Benthem and Hooker with a few modifications here and there suggested by Radlkofer (1883). He has placed *Asteracantha* in the tribe Ruellieæ, which he says is characterised by cystoliths of varying shapes. The haploid chromosome numbers of some members of the family have been determined by Sugiura (Tischler, 1935–37; 1938). Between the classification of Hobein according to cystolith characters and the meiotic chromosome numbers, there seems to exist some correlation. The haploid chromosome number of *Asteracantha* has been found to be sixteen, which number is also found in *Ruellia tuberosa*. Eighteen also appears to be the chromosome number of some other *Ruellia* species. Since 18 and 16 are the haploid numbers of the members of the tribe Ruellieæ, the inclusion of *Asteracantha* in that tribe by Hobein on the basis of cystolith character seems to be justified from a cyto-taxanomical point of view. The chromosome numbers known in this family are too few to warrant a generalisation in regard to classification.

X. Summary

The haploid and diploid chromosome numbers of *Asteracantha longifolia* Nees., are 16 and 32 respectively.

The floral parts develop in the order calyx, andrœcium, corolla, and gynœcium. The anatomy of the stem, root and leaf and the morphology of the thorns have been described.

The development of the micro- and megaspores is traced. In the embryo development suspensor haustorium and micropylar endosperm haustorium have been observed. The endosperm is cellular and the micropylar haustorium is bi-nucleate.

The morphology of the jaculator is described.

In regard to the position of the genus in the family correlation between Hobein's classification on the basis of cystolith features and the chromosome numbers is suggested.

XI. Acknowledgement

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STUDIES IN FLORAL ANATOMY

Part I. Gynæceum Constitution in the Cruciferæ[1]

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Introduction

THE nature of the Crucifer gynæceum was a subject of much discussion in the past and some bitter controversies have been aroused once again during recent years over the number of carpels of which it is composed. A complete review of all the views and hypotheses proposed from time to time on the question seems to be unnecessary since this ground has already been covered by other authors (see particularly Eames and Wilson, 1928 and Bancroft, 1935). It should suffice to say that till recently the bi-carpellary theory was accepted by most botanists in spite of certain obvious difficulties in explaining the nature of the septum which bisects the ovary and the abnormal position of the stigmas which are situated not on the mid-ribs of the two carpels but on the commissures.

Attracted by the appearance of some abnormal ovaries of *Matthiola incana*, Miss Saunders (1923) made a fresh and detailed study of many crucifers and adduced evidence to show that the crucifer ovary consists of four members—two sterile "valve" carpels and two fertile "solid" carpels—, and that the septum, dividing the ovary chamber into two loculi, is true carpellary tissue, formed by the inward extension and subsequent fusion of the two fertile carpels.

Eames and Wilson (1928, 1930) have brought forward much anatomical evidence in support of this view. They hold that the carpel is fundamentally a three-trace organ and that the vascular supply of the two solid carpels is of the same nature as that of the valve carpels which are here sterile. The septum is believed to have been formed by an expansion of the ventral margins of the folded solid carpels. The ovules are extra-carpellary and are supposed to have been extruded out in phylogeny through the walls of the carpels.

[1] Part of the thesis submitted in partial fulfilment of the requirements for a D.Sc. Degree of the Agra University.

On the other hand, Mrs. Arber (1931 *a*, *b*), from her work on the Cruciferæ, concludes that "the anatomical basis on which the quadri-carpellary theory rests, does not stand examination, and moreover this theory introduces unnecessary complexities from which the bi-carpellary theory is free".

Miss Spratt (1932) also supports the bi-carpellary view, but she delimits the carpels differently and is of the opinion that the ovules are borne on their mid-ribs and not on the margins. In a few cases she fails to find any vascular tissue in the two valve positions in young gynæcia of certain crucifers. In her opinion the mid-rib bundles are too important to be missing. Consequently the replum bundles, which according to the quadri-carpellary theory represent the mid-rib bundles of the solid carpels, are here regarded as the dorsal bundles of the two carpels and the ovules as being borne on them.

Eggers (1935) also disagrees with both Saunders and Eames and Wilson and writes: "Jedenfalls haben wir keinen Grund, aus dem Verhalten des Leitgewebes in der Schote auf deren Vierfruchtblättrigkeit zu schliessen".

On the other hand, Dickson (1934), who has made a thorough study of the Papaveraceæ, is in general agreement with the quadri-carpellary theory as conceived by Eames and Wilson.

It was this bewildering divergence in views and interpretations and at times even in the matter of observations that prompted me to study the question afresh. As also pointed out by Arber (1931 *a*), it was experienced that free-hand sections are of little real value in such studies. The material was, therefore, embedded in paraffin and cut on the microtome. The sections were stained with crystal violet and erythrosin since this combination brings out even the smallest bundles with considerable clearness.

The plants studied here fall into five different tribes of the family (Schulz, 1936). They will now be described in regular sequence and such observations as throw light on the morphology of the gynæceum will be discussed at some length.

Investigation

Brassiceæ

(i) *Brassica campestris* L. var. *sarson* Prain.—Fig. 1, A, shows a section of a flower bud at the point where traces for the four median stamens (St.) are passing out (those for the other two having departed earlier) and the whole vascular ring has broken up into two median segments, the replum

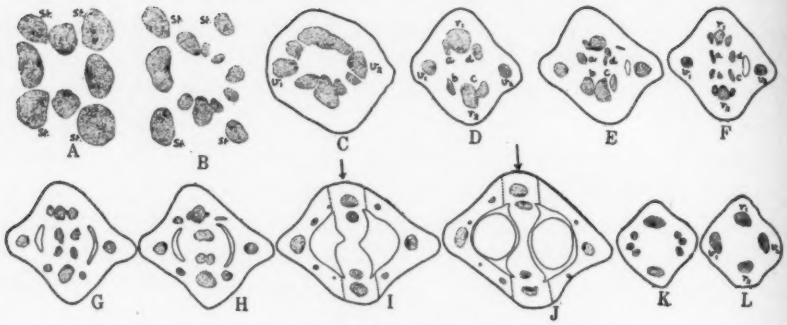

FIG. 1, A–L

T.s. of ovary of *Brassica campestris* (× 20). For explanation see text.

N.B.—Dotted lines in this as well as in the subsequent figures mark the limits of the carpels according to the tetracarpellary theory supported here whereas the arrows when produced towards the centre would delimit the two carpels on one side according to the bi-carpellary view.

strands, and two lateral segments, the valve strands.[2] A little higher up these four divide to form a more or less complete ring of vascular tissue (Fig. 1, B). From the positions of the valve strands one trace now passes out on each side as the median bundle of the valve (Fig. 1, C, bundles v_1, v_2). Out of the six bundles now left in the stele *a* and *d* proceed inward to approach the corresponding bundles *b* and *c* coming from the opposite side (Fig. 1, D–F). Without meeting in the centre they swing in and fuse in pairs to form the inversely oriented placental bundles on the inner side of the replum bundles (Fig. 1, G–I).

Just when *a*, *d* and *b*, *c* are passing in, one small bundle is cut off on each side of each replum strand. This passes out into the corresponding arm of the valve. The remainders of the replum strands constitute the replum bundles just referred to (Fig. 1, I).

Higher up in the style the two lateral bundles in each valve approach the median bundle and finally fuse with it (Fig. 1, K, L). These two bundles thus formed together with r_1 and r_2 (Fig. 1, L) continue right up to the base of the papillose stigma.

(ii) *Brassica oleracea* L. var. *gongylodes* L.—The vascular anatomy of the gynæceum of this species presents some significant variations with regard to the origin of the lateral bundles of the valves. Here, some of the bundles flanking the gaps caused by the passing out of the median bundles

[2] The expressions "replum strands" and "valve strands" are used here in a purely topographical sense and without any previous commitment to any of the two existing theories.

of the valves cut off one bundle each for the corresponding arm of the valve before passing in. In Fig. 2, C–E the bundles *a*, *b* and *d*, for instance, have

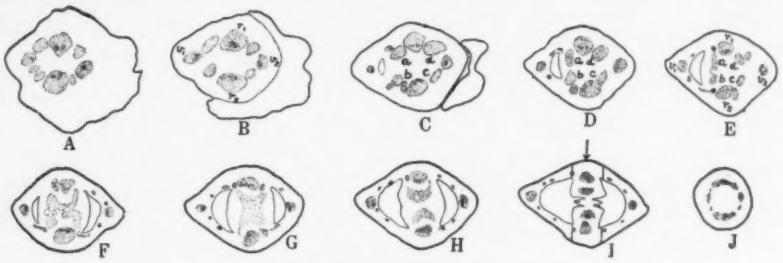

FIG. 2, A–J

T.s. of ovary of *Brassica oleraceæ* (× 20). For explanation see text.

cut off small portions for the margins of the valves but the bundle *c* passes in without doing so and consequently on this *c* side the first lateral bundle for the valve is cut off from the replum strand in the same manner as in the last species.

The remnants of *a*, *b* and *d* and the bundle *c* now proceed toward the centre and fuse there to form an X-shaped plexus of vascular tissue in which the component bundles cannot be distinguished (Fig. 2, F–G). Two groups of vascular tissue emerge out of this plexus in the antero-posterior plane (Fig. 2, H). They have their xylem elements facing those of the replum bundles on their outer side. They give out the ovular traces and are, therefore, the placental bundles. Though their components are not distinguishable there can be no doubt about their having been formed in the usual manner by the fusion of the marginal bundles. Many cases can be cited where due to an *early* fusion of the marginal bundles the placental strands appear as single bundles even at the point of their origin (see among others Eames, 1931; Dawson, 1936; Puri, 1939).

Fig. 2, J shows the vascular ring in the upper part of the style. Higher up only two bundles are left in the positions of the replums and they supply the stigma.

(iii) *Brassica rugosa* Prain.—In this species a ring of procambial tissue with only four xylem groups enters the base of the ovary (Fig. 3, A). The two lateral groups of xylem elements separate off as definite vascular bundles v_1 and v_2, which pass out as median bundles of the valves (Fig. 3, B). The remaining two arcs of vascular tissue, *i.e.*, the replum strands, occurring in the antero-posterior plane, contract a little, as it were, and become more concave on the inner side (Fig. 3, C) and cut off small bundles at either end

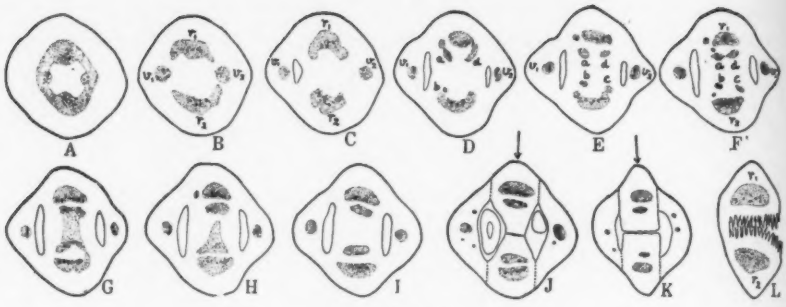

FIG. 3, A–L

T.s. of ovary of *B. rugosa* (× 20). For explanation see text.

(Fig. 3, D–E). While passing in the bundles *a* and *b* (Fig. 3, D) have left small portions on the periphery. In Fig. 3, F the bundles *a* and *d* have come to lie very close together on the inner side of r_1. Similarly the bundles *b*, *c*, occupy positions on the inner side of r_2. Immediately all the four bundles, *a*, *b*, *c* and *d*, are connected by a very light expanse of vascular tissue running across the centre (Fig. 3, G). This central expanse is, however, much fainter than the tissue at its two ends where the separate bundles have lost their individuality to produce the placental bundle seen in Fig. 3, H–I. In Fig. 3, H the central plexus has broken off from one of the placental bundles while in the next the whole of it has disappeared completely.

In some cases it has been observed that no central plexus is formed but the bundles cut off from the replum strands *directly* form the placental bundles.

It should be noted that in this species the valves do not receive any lateral bundles. In some cases very small traces are cut off but they disappear very early.

(iv) *Eruca sativa* Gars.—The passing out of the traces for the median stamens (St.) leaves only four stelar bundles in the receptacle (Fig. 4, A). A little higher up the latter divide to form a more or less complete ring of vascular tissue (Fig. 4, B). From the positions of the valve strands one trace now passes out on each side as the median bundle of the valve (Fig. 4, C, bundles v_1, v_2). The bundles *a*, *b* and *c*, *d* lying on either side of the gaps thus formed approach one another and the vascular ring is completed once again, but this time it is somewhat laterally compressed (Fig. 4, D–E). Fresh gaps are soon formed at the four corners of this ring due to the passing out of the lateral traces into each arm of either of the valves (Fig. 4, E–F).

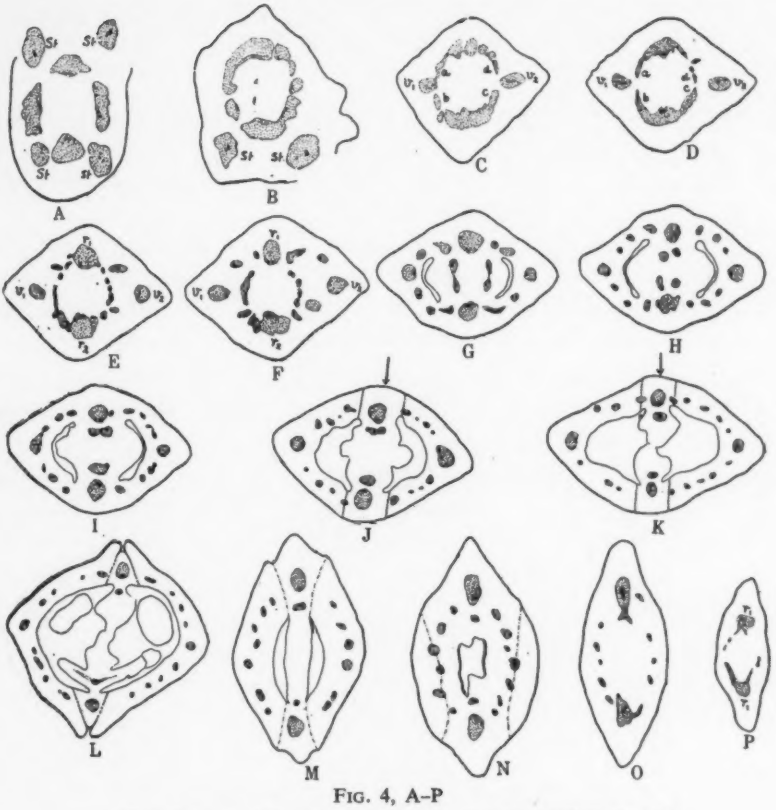

FIG. 4, A–P

T.s. of ovary of *Eruca sativa* (× 26). For explanation see text.

Due to the disappearance of some of the vascular tissue, the lateral bridges, which connect the two replum strands r_1 and r_2, gradually fade away and finally four bundles emerge out of them (Fig. 4, G). These swing inward in opposite directions in such a way that they come to lie in pairs just on the inner side of the replum bundles (Fig. 4, H). Here they fuse to form the placental bundles with inverse orientation (Fig. 4, I).

In Fig. 4, J, small protuberances of tissue are seen marked off, one on each side in the middle of the septum. A little higher up both of them become detached and gradually disappear. It is quite likely that these are the degenerating glands of the type very often seen in the axils of, or alternating with, the stamens (see Arber, 1931 *a* and Norris, 1941). The residual septum then becomes deeply constricted in the middle (Fig. 4, K). Fig. 4, L

shows the condition as seen in the middle of the ovary where the valves have over-grown, as it were, to cover the replum. The ovular traces arise from the placental bundles.

Higher up towards the style, the valves decrease in size and the replums show a corresponding increase (Fig. 4, M–N). The style and stigma are thus composed only of the replum tissue (Fig. 4, O). The stigma receives only the two replum bundles (Fig. 4, P).

(v) *Raphanus sativus* L.—This species has already been studied by several authors and the observations made here tally with those of Arber (1931 *a*). However, there is some difference in the interpretation, and hence I consider it worth-while to summarise the results very briefly.

Fig. 5, A–B, represent the condition during and after the departure of the stamenal traces (St.). Soon after the passing out of the median bundles

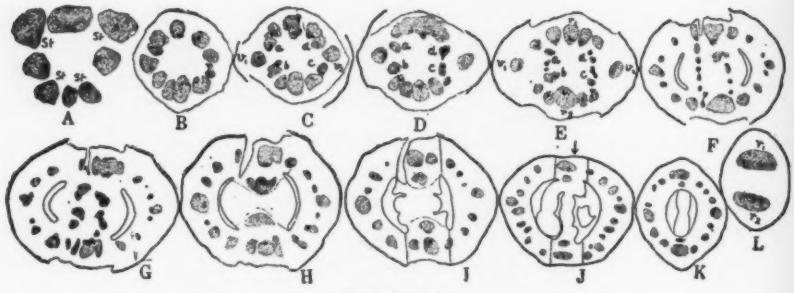

FIG. 5, A–L

T.s. of ovary of *Rephanus sativus* (× 26). For explanation see text.

of the valves the smaller bundles *a*, *b* and *c*, *d* flanking the gaps, begin to approach one another towards the centre (Fig. 5, C–D). They branch and anastomose and with the help of some other very small bundles, cut off from the replum strands, almost close the gaps caused by the median bundles of the valves (Fig. 5, E–F). Just when this ring is being completed new gaps are formed by the passing out of lateral traces for each arm of the valves. Then the median strands constitute the replum bundles and four main bundles emerge out of the remaining tissue of the stele (Fig. 5, G). These four bundles swing in to their positions on the inner side of the replum bundles and finally fuse together to form the inversely oriented placental bundles (Fig. 5, H).

The septum presents the same appearance as in the last species (Fig. 5, I). The stigma receives only the two replum bundles (Fig. 5, L).

It is interesting to note that as we approach the stigma the size of the vasuclar bundles slightly increases. This is a feature common to many of the species studied by me. Arber (1931 *a*), who also observed it, thinks that this may be "connected with the hindrance to the further flow of the sap, when the distal region of the organ is approached". As pointed out by Hunt (1937), it is also likely that they may refer to the massive stigmas once possessed by these species or their ancestral forms, now lost during evolution.

Lepidieæ

(1) *Iberis odorata* L.—In this species the origin of the placental bundles could not be made out in young gynæcia (Fig. 6, A–G). In older ones,

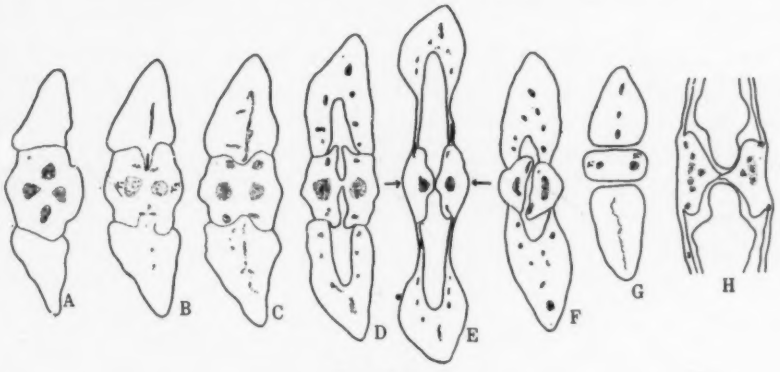

FIG. 6, A–H

T.s. of ovaries of *Iberis contracta* (× 20). A–G, young ovary; H, older ovary. For explanation see text.

however, an inversely oriented placental bundle is visible on the inner side of each replum bundle (Fig. 6, H). It may be on account of great reduction that the placental bundles do not get distinctly separated from those of the replums. But it seems pretty certain that their mode of formation is, in all essential respects, similar to that found in above-described species.

(ii) *Capsella bursa-pastoris* (L.) Medikus.—As usual there are four bundles entering the base of the ovary. A little higher up the replum strands cut off one bundle on either side. Out of the total of eight bundles thus formed v_1 and v_2 now pass out to become the median bundles of the valves (Fig. 7, A). The bundles *a*, *d* and *b*, *c* then proceed toward the centre and fuse there into an X-shaped plexus (Fig. 7, B–C). Later on the plexus breaks up into two parts each of which lies on the inner side of the replum

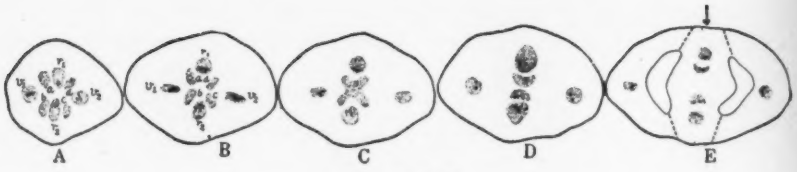

FIG. 7, A–E

T.s. of ovary of *Capsella bursa pastoris* (×40). For explanation see text.

bundle. This is the placental bundle with the usual inverse orientation (Fig. 7, D–E).

Arabideæ

(i) *Cardamine hirsuta* L.—This species represents a comparatively simpler condition. The passing out of the median bundles of the valves, v_1, v_2, leaves only six bundles in the stele (Fig. 8, A). Of these the median

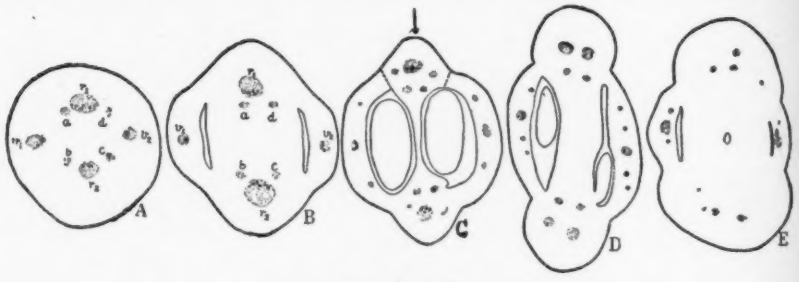

FIG. 8, A–E

T.s. of ovary of *Cardamine hirsuta* (× 40). For explanation see text.

ones, r_1, r_2, form the replum bundles while those lying on the sides swing slightly inward and take up positions on the inner side of the replum bundles (Fig. 8, B). They remain distinct throughout and supply ovular traces separately (Fig. 8, C).

Higher up each of the replum bundles divides into two (Fig. 8, D). Towards the apex the bundles of the valves and the replums decrease in size (Fig. 8, E) and finally disappear almost simultaneously.

Matthioleæ

(i) *Matthioloa annua* (L.) S W.—Fig. 9, A, shows the four bundles entering the base of the ovary. They branch and soon form more or less a complete ring (Fig. 9 B–C). After the passing out of v_1 and v_2 the smaller bundles *a* and *d* approach *b* and *c* coming from the other side (Fig. 9, D–E).

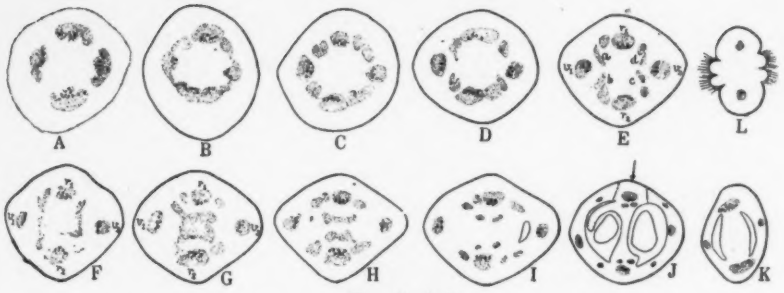

FIG. 9, A–L

T.s. of ovary of *Matthiola annua* (× 26). For explanation see text.

They fuse in the centre to form an X-shaped plexus (Fig. 9, F–G). In Fig. 9, H, the plexus has lost some of its central tissue and has split up into two parts, each of which again loses its central portion and results in two bundles lying right and left inside the replum bundles (Fig. 9, I). They remain quite distinct throughout their course and give out ovular traces.

In this species the lateral bundles of the valves, if present, are cut off from the replum strands after those which form the central plexus (Fig. 9, I–J).

The style receives only four bundles out of which the replum bundles are more prominent than the median bundles of the valves (Fig. 9, K). Fig. 9, L represents the six-lobed stigma which receives the two replum bundles only. The lateral lobes of the stigma are, however, very small and are upward continuations of the valves.

Hesperideæ

(i) *Cheiranthus cheiri* L.—The course of events in this species is illustrated in Fig. 10, A–K. The behaviour of the bundles *a*, *d* and *b*, *c* is more

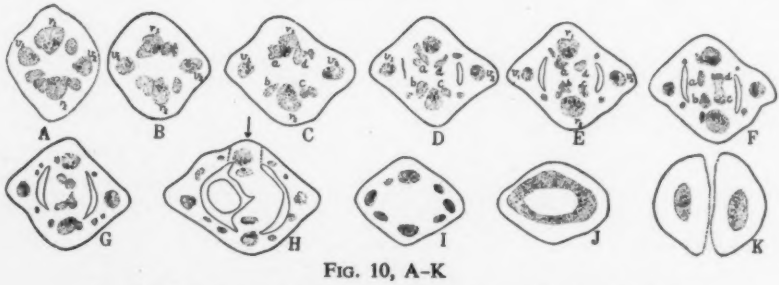

FIG. 10, A–K

T.s. of ovary of *Cheiranthus cheiri* (× 26). For explanation see text.

or less similar to that in *Brassica rugosa*. In Fig. 10, F the bundles *d* and *c* are seen connected together by a short expanse of vascular tissue. In the next figure this 'bridge' has disappeared and there are left two bundles on the inner side of each replum bundle (Fig. 10, G). A little later they fuse into placental bundles (Fig. 10, H).

Fig. 10, I shows eight bundles passing into the basal region of the style. Higher up all the bundles anastomose to form a complete ring of vascular tissue (Fig. 10, J). As the stigma is reached the latter breaks up into two arcs which finally resolve into two bundles occurring in the positions of the replum bundles (Fig. 10, K).

In addition to these *Cherianthus allionii*, *Nasturtium officinalis*, *Senebiera pinnatifida*, *Lepidium officinalis* are some of the other species examined but they all show the same ground-plan as described above and hence it is no necessary to deal with them separately.

Discussion

(i) *Ground-plan of the Vascular Supply of the Gynæceum*

In all the plants studied here four bundles usually enter the base of the ovary. A little higher up they give out small branches on either side and tend to form a ring of vascular tissue. From this there arise four distinct systems of vascular bundles, two median and two lateral. Each of these typically comprises three bundles—one mid-rib and two laterals. In the median system the lateral bundles often swing inward and fuse to produce inversely oriented placental bundles.

These four systems are, however, to be clearly distinguished from the replum and valve strands with which they may not necessarily correspond.

(ii) *Nature and Number of the Carpels*

(*a*) *The Bi-carpellary Theory.*—According to this view the Crucifer gynæceum is composed of two carpels with a valve-like contour. The two smaller areas with their inward prolongations represent the carpellary margins which are believed to have become hypertrophied due to their ovule-bearing function.

The chief objection to this interpretation has always been that in most genera the stigmas are situated over the commissures and not over the mid-ribs of the carpels the normal position for the stigmas.

The method of dehiscence, apparently neither loculicidal nor septicidal, is also difficult to explain on this interpretation.

Further, the nature and behaviour of many of the bundles entering the base of the ovary cannot be satisfactorily explained on the basis of this interpretation.

The exponents of this theory regard the median bundles of the lateral systems the mid-rib bundles of the carpels and the three bundles of the median system (two after fusion) to be branches of the same placental bundle. There is however, no justification for such a presumption since it has been shown that in most of the species described above the marginal bundles of the median systems have no direct connection, whatsoever, with its median bundle.

Then, in what manner the inverse orientation of the bundle supplying ovular traces is brought about has also failed to receive a satisfactory explanation.[3] Merely suggesting that the inversion is a physiological necessity (Eggers, 1935) does not, in any way, carry us nearer the solution of the problem.

As pointed out before, Spratt (1932) has suggested different limits for the two carpels and holds that the ovules are borne on the mid-ribs of the carples and that the placental bundles are simply branches cut off from the mid-rib bundles for supplying ovular traces. It may be pointed out in this connection that ovules are so far known to be borne only on the margins of the carpellary "leaves" and there appaers to be no special reason why the Crucifers should be treated as exceptional by assigning their ovules to the mid-rib. Moreover, some of the other objections mentioned above also apply to this interpretation.

(*b*) *The Tetra-carpellary Theory*.—According to this view there are four carpels in the Crucifer gynæceum, occurring in the same whorl or in two different whorls of two each. The so-called valves of the ovary make up the sterile carpels while the segments between the valves constitute the fertile and solid carpels. During recent years Saunders (1923, 1937) on one hand and Eames and Wilson (1928, 1930), on the other, have been great exponents of this view. They, however, differ considerably in detail and it is, therefore, desirable to deal with their conceptions separately.

The Conception of Saunders.—According to her the four carpels occur in the same whorl and every one of them is individually represented by one of the four bundles entering the base of the ovary. The lateral carpels, which are of the "valve" type, are always sterile while the median ones are

[3] Arber (1938) has attempted to explain it on "mechanical grounds" in some of the Papaveraceæ but that interpretation cannot be applied to the Cruciferæ.

of the " consolidated " type and are always fertile. The latter show the following features: (1) They bear a row of ovules on either side of the mid-rib. (2) They possess a pair of well-developed bundles close to the mid-line, which furnish traces to the ovules. (3) They bear the stigmas which need not be considered now as commissural.

The " consolidated " type of carpel is believed to appear in two forms which, however, intergrade with one another. When contracted to a column or radial sheet of tissue, as is the case in ovaries developing into siliqua fruits, it is conveniently distinguished as a " solid " carpel; in its most reduced form it is believed to be composed merely of a fibro-vascular cord. When expanded, so that in outward shape it resembles the valve type, it is said to be " semi-solid ", as is the case in ovaries forming the silicula type of fruit.

Such an enunciation of the tetra-carpellary theory has been subjected to much adverse criticism by Arber (1931 *a*, 1931 *b*), Eames (1931) and Eames and Wilson (1928, 1930). Without going into details I will simply refer to some of the more important objections raised by these authors.

By tracing the course of the vascular bundles in serial sections Arber (1931 *b*) has shown beyond doubt that the bundles seen in the base of the ovary do not always correspond to the four bundles seen higher up in it. She has also made it clear that the placental bundles are formed by a contribution from both the valve and replum strands and concludes (Arber, 1931 *b*, p. 178) that " this interdependence of the valve and replum bundles seems irreconcilable with E. R. Saunders' interpretation of them as belonging to different carpels ".

Referring to the " semi-solid " carpels of Miss Saunders, she writes: " In a typical narrow siliqua, each valve which becomes detached on ripening is described as consisting of one sterile lateral carpel alone, whereas each valve which falls from the silicula of *Lunaria* is described as representing one much reduced sterile lateral carpel, together with one half of each of the two fertile semi-solid median carpels. But it will be recognised on comparing—a transverse section of a very young ovary of *Lunaria annua* L.—with a corresponding section of any typical Crucifer with narrow siliqua—that they are essentially identical; to explain the nature of the valve on different lines is wholly arbitrary and unconvincing " (Arber, 1931 *a*, p. 38).

The Conception of Eames and Wilson.—Though Eames and Wilson also uphold the tetra-carpellary theory, their method of approach to the subject is fundamentally different from that of Saunders. According to them the four carpels are arranged in two whorls of two each. The outer whorl is

made up of two valve-like open carpels bearing no ovules. The inner whorl consists of two solid or closed ovule-bearing carpels in which the loculus has disappeared by gradual reduction. They have based this interpretation mainly on anatomical grounds. The solid carpels have fundamentally the same vascular supply as the valve carpels with the only difference that the ventral bundles of the former often fuse together to form an inverted placental bundle which supplies traces to the ovules.

Such a view not only gives a convincing explanation of the peculiarities of the vascular supply of the gynæceum but also meets the various objections levelled against the bi-carpellary theory. Every one of the four systems seen in the base of the ovary (see p. 176) thus represents the vascular supply of a single carpel.

The present writer, although sharing the views of Eames and Wilson on the nature of the carpels, finds some difficulty in agreeing with them in the details involving the origin and behaviour of the ventral bundles of both the valves and solid carpels. It is, therefore, proposed to discuss this point at some length; for it is not only important in determining the nature of the carpels but will also throw some light on the nature of the septum and the position of the ovules.

The Marginal Bundles of the Valve Carpels.—Generally the marginal bundles of the valve carpels arise from the sides of the replum bundles when

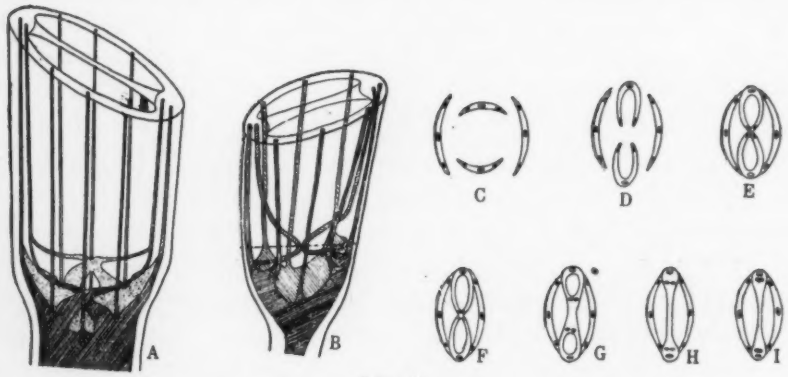

FIG. 11

A.—Theoretical diagram to illustrate the vascular ground-plan of the *Crucifer gynæceum.*

B.—After Eames and Wilson's (1928) Fig. 5 to show their conception of the *Crucifer gynæceum.* According to the interpretation suggested here the bundles passing in towards the centre have to be regarded as stelar bundles up to the level of the dotted line and not as ventral bundles of the solid carpels.

C–I.—Reproduction of Eames and Wilson's (1930) Fig. 5 to show the mode of solidification of the carpels.

the four smaller bundles, *a*, *b*, *c* and *d* (see Figs. of *Brassica campestris*, *Raphanus*, *Eruca*, etc.) have left for the centre. Arber (1931 *a*) has also reported a similar condition in *Brassica campestris* and some others. But Eames and Wilson do not draw any attention to this fact. On the other hand, they appear to suggest in a general way that the bundles lying on either side of the mid-rib bundles of the valve carpels pass out as their marginal bundles (see Eames and Wilson, 1928, Fig. 5, reproduced here as Fig. 11, B).

In some cases they may arise directly from the bundles *a*, *b*, *c* and *d* before the latter pass inward (see Figs. of *Brassica oleracea*, *Cheiranthus cheiri*, etc.). In still other cases the marginal bundles of the valve carpels may be entirely absent as in *Iberis* and *Capsella* or they may be visible at the base and disappear a little higher up as in *Matthiola* and *Brassica rugosa*.

The Marginal bundles of the Solid Carpels.—Since these are the last traces to arise and the receptacular stele does not usually continue beyond the level of their origin to an appreciable extent, it becomes very difficult to determine the exact place of origin of the marginal bundles of the solid carpels.

Eames and Wilson (1928, pp. 254–55) have described a wide range of behaviour for these bundles:

(1) In many species they are said to arise from the sides of the mid-rib bundles of the solid carpels and then turn in and fuse with each other. The bundle so formed approaches the corresponding of the opposite carpel and meets it in the centre of the ovary. Then the two swing outward and take their positions just inside of the dorsal bundles (see Fig. 5 of Eames and Wilson, reproduced here as Fig. 11, B).

(2) In some cases " they may not fuse but approach the centre of the ovary and then retreat to the position just inside of the dorsals, meanwhile remaining distinct ".

(3) " Again, all four such traces may meet in the centre and form a somewhat confused mass of vascular tissue in which the component parts are distinguishable with difficulty."

(4) " In other cases the ventrals of each carpel fuse without passing far toward the centre of the ovary, and then swing slightly backward and follow up inside the dorsals."

(5) " In a few species the ventrals, after fusing near the centre of the ovary split apart again and swing back, taking position to the right and left inside of the dorsals."

I am unable to confirm the first-named behaviour although this is reported to be the commonest. The nearest approach to it, that I could find, was in *Brassica rugosa* where the fusion products of *a*, *d* and *b*, *c* respectively are connected by a small bridge of vascular tissue. But in order to fit this example in the case in question we have to regard the bundles *a*, *b*, *c* and *d* as the marginal bundles of the solid carpels which is not correct.

In the third-named behaviour the four marginal bundles are said to fuse together in the centre to form a confused mass of vascular tissue. If this be so it would mean that the whole of the receptacular tissue has disappeared even at such low a level as the origin of the marginal traces. As long as we believe that carpels are appendicular organs (Prof. Eames himself is a strong exponent of this view) we must presume that the tissue of the receptacle and in many cases even the vascular supply of the same, must continue for some distance (say a few microns) above the origin of the carpellary traces if not above the carpels themselves.[4] The contention that the ventral bundles of the opposite carpels fuse together in the centre so near the level of their origin, is a complete negation of this apparently logical and reasonable presumption. Besides, in cases like *Coringia orientalis*, *Cheiranthus allionii*,[5] etc., (Eames and Wilson, 1930, p. 651) even the vascular tissue of the receptacle continues for some distance after the origin of the ventral traces of the solid carpels, to say nothing of the ordinary receptacular tissue which must necessarily end at a still higher level than the last traces of its stele.

The fifth type of behaviour also does not appear to be quite convincing. In determining the nature of the carpels, Eames and Wilson have depended entirely on the behaviour of the marginal bundles and have always regarded them as representing the course and behaviour of the margins of the carpels. If the case under reference is interpreted in this light it should mean that the carpels first became closed since the ventrals had fused and then opened again allowing the ventrals to split apart! This, however, is highly improbable.

These are the difficulties which one encounters if the views of Eames and Wilson are to be accepted *in toto*. All of them are evidently due to their mistaking the stelar bundles *a*, *b*, *c* and *d* for the marginal bundles of the solid carpels. In a later communication (Eames, 1931) Eames has devoted

[4] Arber (1937) has brought together interesting evidence about the prolongation of the receptacular tissue beyond the origin of the carpels.

[5] With the herbarium material of this species, which alone was available to me, I could not confirm this point.

considerable attention to the question, "When is a trace not a trace but a receptacular bundle?", and there he has clearly shown that in *Illicium anisatum* the two bundles left after the passing out of the mid-rib bundles of the carpels cannot technically be regarded as marginal bundles although for all intents and purposes they *are* marginal bundles. He seems to have overlooked the fact that a similar condition exists in the Cruciferæ.

I shall now pass on to explain the view to which I have been led as a result of my study of the Crucifer gynæceum. I maintain that the bundles *a*, *b*, *c* and *d* in the species described above are true stelar bundles and that it is from these that the marginal traces of the solid carpels are derived. If they are themselves regarded as marginal bundles then the later origin of the marginals of the valve carpels from between them and the replum bundles (the mid-rib bundles of the solid carpels) cannot be justified. I have recognised five types of behaviour of these bundles before they give rise to *true* marginal traces. These can, however, be regarded only as convenient classes which are not separated from one another by any hard and fast rules, but which, nevertheless, cover all the possible variations in the behaviour of the bundles in question.

(1) In *Eruca sativa*, when the dorsal traces for the valve carpels have made their exit, the bundles *a*, *b* and *c*, *d* approach one another. During their inward course they branch, anastomose and with the help of some other bundles cut off from the sides of the replum strands complete the vascular ring again. From the sides of this ring marginal traces for the valve carpels are given out and the replum bundles become the dorsal traces of the solid carpels. Out of the remaining tissue then there emerge out four bundles which swing in pairs in opposite directions as the marginal bundles of the solid carpels and ultimately fuse in pairs as the inverted placental bundles.

In *Raphanus sativus* also the bundles *a*, *b*, *c* and *d* exhibit a similar behaviour.

(2) In *Brassica campestris* the bundles *a*, *b*, *c* and *d* proceed toward the centre and without undergoing any fusion they (or remains thereof, for some of the tissue must have disappeared in their upward course) swing backward and inward as marginal bundles of the solid carpels and fuse in pairs just on the inner side of the mid-rib [*cf.* case (2) of Eames and Wilson].

(3) In *Capsella* also the bundles *a*, *b*, *c* and *d* proceed inward but here they fuse to form an X-shaped plexus of vascular tissue which for reasons already discussed can only be regarded as stelar in nature. Very soon the plexus breaks up into two groups, each constituting an inverted placental

bundle on the inner side of the mid-rib bundles of the solid carpels. *Brassica oleracea*, *B. rugosa* and *Cheiranthus cheiri* all show slight variations of the same type [*cf.* case (3) of Eames and Wilson].

(4) In *Matthiola* also the bundles *a*, *b*, *c* and *d* fuse into an X-shaped plexus of vascular tissue which breaks up into two parts by losing its central portion. The marginal bundles, though differentiated, are still connected together for some distance by very small portions of the receptacular tissue which soon disappears leaving two distinct bundles, one on either side of each of the mid-rib bundles belonging to the solid carpels [*cf.* case (5) of Eames and Wilson].

(5) *Cardamine* appears to represent an extreme case where the bundles *a*, *b*, *c* and *d* do not *apparently* carry any stelar tissue with them. They swing slightly inward and take up their positions as the marginals of the solid carpels.

Thus in all cases the bundles *a*, *b*, *c* and *d* are true stelar bundles. It is only after their stelar part of tissue has disappeared that they can be regarded as true marginals. (See foot note 6.)

(iii) *Solidification of the Carpels and Position of the Ovules*

The inverted state of the placental bundles—a condition met with only in the multicarpellary gynæceum with axile placentation—indicates very clearly that the carpels, although without loculi, are of the closed type, in which the margins have turned in and fused opposite to the mid-rib bundles. Hence it is concluded that the median carpels in the Cruciferæ are solid.

Eames and Wilson (1930) have given a series of diagrams to show the way in which this solidification of the carpels might have occurred (see their Fig. 5, reproduced here as Fig. 11, C–I). There is, however, some difficulty in accepting their suggestion *in toto*. The figures referred to above may be interpreted to mean that the carpels have suffered only lateral contraction; for while the margins of the solid carpels remain joined together in their original position and maintain the same distance from the mid-rib, the placental bundles are made to shift outward. This moving out of the placental bundles has been regarded by them as a strong evidence for a contraction of the carpels. It is, however, difficult to understand a contraction which can shift the marginal bundles from the centre to the periphery without affecting the margins themselves.

Such an interpretation is, of course, unavoidable if we accept that the septum is carpellary in nature (Eames and Wilson, 1928). But it must be pointed out that this introduces a serious inconsistency in the tetra-carpel-

lary theory, for when it comes to explaining the nature of the median carpels the course and position of the ventrals are regarded as absolute guides to the course and position of the carpellary margins (Eames and Wilson, 1928, p. 264), but here the two structures are described to have no apparent relation with each other.

With regard to the ovules these authors had to suggest, although " reluctantly ", that they have extruded out by penetrating through the wall of the carpels. This is the only possibility if the marginal bundles and consequently the margins of the solid carpels are supposed to fuse in the centre. Spratt (1932) appears to have discarded the tetracarpellary theory of the Crucifer gynæceum merely on this account. She does not think it likely that such tender structures as ovules could ever have made their way out of the carpellary walls.

The mode of solidification of the carpels suggested here in Fig. 12, A–L. The writer believes that the extrusion of the ovules took place before the

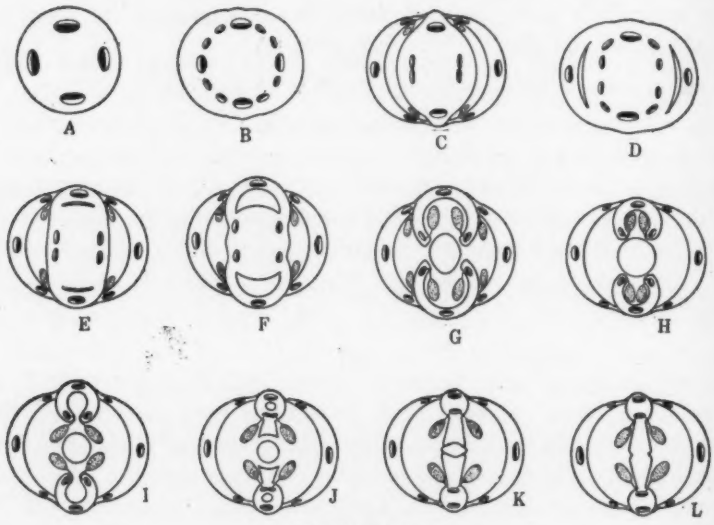

FIG. 12, A–L

Theoretical diagrams to show the author's conceptions of the mode of extrusion of the ovules, solidification of the carpels and formation of the septum.

margins of the carpels fuse together to close the loculus and that the ovules were pushed out of the loculus—probably due to lack of space—through the open margins into the cavities enclosed by the valve carpels. It was only after extrusion of the ovules that the margins of the fertile carpels fused

together (Fig. 12, I–J). Further consolidation resulted in complete elimination of their loculi and the carpels became solid (Fig. 12, K).[6]

(iv) *Nature of the Septum*

According to Eames and Wilson the septum is carpellary in nature' *i.e.*, it is formed by the fusion of the margins of the two solid carpels. The main evidence, and probably the only one, on which this view is based is the supposed meeting of the ventrals of the solid carpels in the centre.

In the first place it has already been shown that the marginal bundles do not meet in the centre and what are believed to be ventrals are really stelar bundles (see foot note 6 below) so that the tissue in which they run cannot be regarded as other than receptacular.

Secondly, even if it is accepted that the bundles in question are really the ventral bundles of the solid carpels, their outward movement towards the mid-rib bundles must lead to the conclusion that the margins of the solid carpels have also receded (for the behaviour of the ventrals cannot but be regarded as representing the behaviour of the carpellary margins themselves). The septum could then arise only by out-growths from the margins of the solid carpels.

The orthodox view that the septum is false still seems to be most satisfactory. In the basal region it is mainly receptacular but as we go upward the axile portion goes on decreasing and the placental in-growths go on increasing until they fuse together in the centre. However, these in-growths arise from the margins of solid and not from the fused margins of the valve carpels as the orthodox view demands.

Eames and Wilson's main objection against the false nature of the septum is best put in their own words (Eames and Wilson, 1928, pp. 251–52):

" If the dissepiment is false, that is of placental origin and not morphologically formed from the carpel walls then the placentæ are normal parietal placentæ and the ovules are borne in a loculus falsely divided into two chambers. Anatomical evidence, already used by earlier students, and again brought forward by the writers, shows that the ovules are not attached to the placentæ in the manner in which ovules are normally borne on parietal placentæ. The placentæ are clearly not parietal and the above interpretations are not correct."

[6] In a personal letter Professor Eames has expressed himself as in agreement with my interpretation of the nature of the bundles *a*, *b*, *c* and *d* as also with the mode of extrusion of ovules suggested here.

It is quite apparent that the above objection applies to the false septum as it is conceived in the bicarpellary theory. It does not hold good for the present case where the septum is believed to have been formed by the placental out-growths of the solid carpels (Fig. 12, G–L).

The anatomical data obtained here are so uniform that it is not possible to draw any conclusions concerning relative degrees of evolutionary advancement among the genera whose representatives have been examined by me as also by Arber (1931 *a*, 1931 *b*). The only noteworthy feature is the behaviour of the last stelar bundles which give rise to the marginal traces of the solid carpels. But no importance can be attached to it since in the tribe Brassiceæ alone practically all the variations in the behaviour of these bundles can be noticed.

Summary

A detailed study has been made of the vascular supply of the ovary of about a dozen of species belonging to eight different genera.

The tetra-carpellary theory of the Crucifer gynæceum, as proposed by Eames and Wilson, has been supported and placed on firmer grounds.

A conception, different from the one given by Eames and Wilson, is suggested for the solidification of the carpels and the extrusion of the ovules. The fusion of the margins of the fertile carpels is believed to have taken place only after the ovules had been extruded from the loculus into the cavity enclosed by the sterile carpels.

The septum is believed to be mainly receptacular in the basal region and placental in the upper.

I am thankful to Dr. P. Maheshwari for the help he rendered in the preparation of this paper. I am also indebted to the Director, Royal Botanic Gardens, Kew, for kindly sparing some of his herbarium material for the sake of comparison. My sincere thanks are also due to Prof. A. J. Eames and Prof. R. R. Gates for some helpful criticism.

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A STUDY OF THE VITAL CAPACITY OF MYSOREANS

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The physiological standards in relation to varying age periods have been well-established in Western countries. Of these the importance of standards of vital capacity in health and disease can hardly be over-emphasised. Although physiology is being taught in Indian Medical Colleges and Schools for nearly a century, it is regrettable that no serious attempt at the estimation of normal physiological standards was made until 1929, when Bhatia[1] studied the vital capacity of one hundred medical students and concluded that in Bombay, the vital capacity of students that came under his observation was on an average 3·091 litres. Further he maintained that the vital capacity of Indians was lower than the standard established for Europeans and Americans. Mukherjee and Gupta[2] in 1931 determined the vital capacity of twelve Bengali students. In their opinion the vital capacity of Bengali students is also lower than that of the Western standards. In their study of vital capacity of students in Madras, Krishnan and Vareed[3,4] maintained that the Madras students possess a definitely lower vital capacity than that of Westerners. Whilst Bhatia suggests that the lower vital capacity of his students in Bombay may be due to a definite 'racial factor', Krishnan and Vareed are emphatic in their denial of the influence of the 'racial factor' on vital capacity. In this vast country the vital capacity of students in three widely separated cities on the sea-coast has been found to be definitely lower than the corresponding Western standard. In their studies Bhatia, Mukherjee and Gupta, Krishnan and Vareed followed the classical method first introduced by Hutchinson. In order to elucidate the question whether the vital capacity of students in a city situated about 3,000 feet above sea-level would show any variation from the figures arrived at by workers in India, the present investigation was undertaken at the suggestion of Prof. A. Subba Rau.

Material and Method

The subjects whose vital capacity measurements were made numbered in all 166 (17–35 years of age) of which 160 were medical students and

six were staff members of the Medical College. In all cases the diet and nature of physical exercise taken by them were invariably noted. Standing height measured was without footwear; weight was taken without clothes; sitting height was measured as recommended by Dreyer. The difference in the chest girth, at the level of the nipples, at maximum inspiration and expiration was taken as the chest expansion. The surface area of the body was calculated from the nomogram of Boothby and Sandiford. For measuring the vital capacity the instrument used was Hutchinson's spirometer supplied by Messrs. Baird and Tatlock Company. Three trials in standing position, and at intervals of ten to fifteen minutes were given and the highest of the three values was taken as representing the vital capacity of the individual. In all cases, before the actual measurements were taken, the students were given ample opportunity to familiarise themselves with the technique involved with a view to minimising errors.

Results

The data obtained have been grouped and presented in Tables I–VII; statistical calculations made from the grouped data are embodied in Tables VIII and IX. From these data Figs. 1–7 are drawn and their significance is discussed below.

TABLE I

Vital Capacity Frequency

Vital capacity in litres ..	1·7	2·0	2·3	2·6	2·9	3·2	3·5	3·8	4·1	4·4
Number of persons in each group	3	1	5	20	33	49	29	19	6	1

FIG. 1

Fig. 1 gives the distribution of the vital capacity values of the present series. To test the normality of it γ_1 and γ_2 of Fisher were calculated. The value of γ_1 was found to be $-0{\cdot}1708 \pm 0{\cdot}189$ indicating an asymmetry in which the mode of the series lies at a higher level than the mean; the value

of γ_2 was found to be $-0\cdot099 \pm 0\cdot375$, showing the frequency distribution curve to be narrower at the base than the normal one. Since the probabilities of finding such an asymmetry and flatness were estimated at 82 per cent. and 61 per cent. on such a material as the present one, the assumption that the distribution of the vital capacity values to be a normal one is warranted.

The average vital capacity of these 166 subjects has been calculated to be $3\cdot191 \pm 0\cdot0379$ litres and standard deviation to be $0\cdot4856 \pm 0\cdot2670$ (Table VIII). If the mean and the standard deviation of the present series were to represent a correct estimate of means and standard deviations of an indefinitely large number of such series, then 99 per cent. of the values of vital capacity of this series must lie between 1·734 and 4·648 litres. It is found that all values lie within these limits except in two cases which were found to be due to lack of co-operation on the part of subjects.

TABLE II

Average Vital Capacity according to Age

Age in years	Number of subjects	Average vital capacity in c.c.
17	2	2650
18	5	3120
19	12	3150
20	31	3143
21	33	3300
22	37	3185
23	20	3207
24	12	3158
25	7	2985
26	2	3275
27	1	3400
28	2	3000
32	1	3600
35	1	3300
Average vital capacity		3190

Note.—Omitting the cases referred to in the text,
the average vital capacity for age 22 .. 3270 c.c.
" " " 23 .. 3287 "

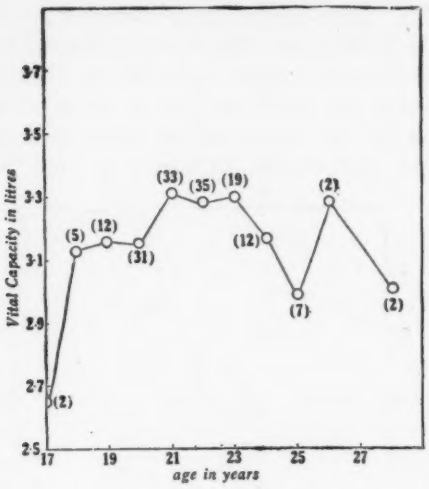

FIG. 2

Fig. 2 shows the relation of vital capacity to different age periods. In Mysore students, the vital capacity is observed to increase with age until it reaches the maximum value at the age of 21 years. Difference of opinion exists about the age at which the maximum of vital capacity is reached. Hutchinson[5] recorded it in the 30th year of life of English people. Stewart[5] noticed the maximum vital capacity in 20 year old American subjects.

TABLE III

Average Vital Capacity according to Standing Height

Height in cm.	Number of persons	Average vital capacity in c.c.
145	1	1700
150	2	2500
155	11	2575
160	36	2909
165	43	3233
170	45	3297
175	22	3561
180	6	3858
Average vital capacity per cm. of height		19·1

Krishnan and Vareed[3,4] found the highest vital capacity in students of Madras at the age of 21 and 23 years in their first and second series. Further, it is observed that the values of vital capacity for ages of 22 and 23 years are also in the neighbourhood of the maximum value in students of Mysore. So it is likely that the maximum of vital capacity is reached in the students of both Mysore and Madras between the ages of 21 and 23 years.

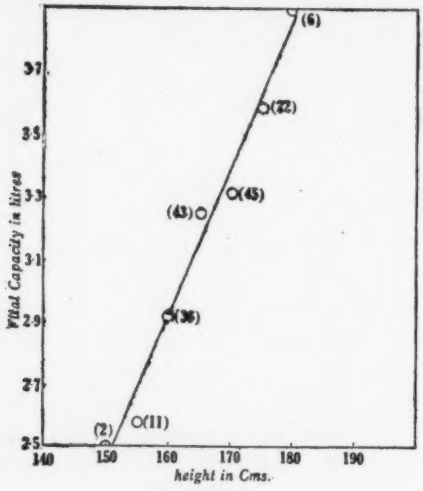

FIG. 3

TABLE IV

Average Vital Capacity according to Weight

Weight in Kg.	Number of persons	Average vital capacity in c.c.
35	2	2150
40	8	2534
45	30	2969
50	60	3202
55	47	3327
60	13	3404
65	5	3380
70	1	4350

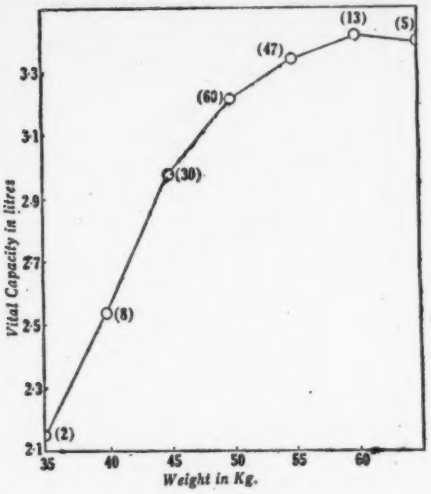

FIG. 4

TABLE V

Average Vital Capacity according to Sitting Height

Sitting height in cm.	Number of persons	Average vital capacity in c.c.
76	1	1700
78	5	2480
80	15	2733
82	17	2856
84	32	3097
86	44	3215
88	29	3447
90	19	3608
92	3	3633
94	1	3550

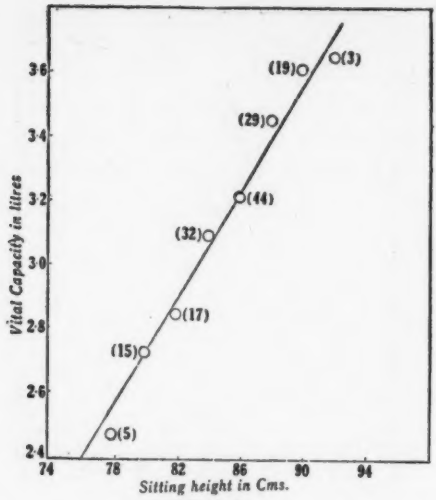

FIG. 5

TABLE VI

Average Vital Capacity according to Surface Area

Surface area in sq. metre	Number of persons	Average vital capacity in c.c.
1·20	1	1700
1·25	3	2533
1·35	6	2628
1·40	10	2750
1·45	17	2828
1·50	28	3114
1·55	31	3234
1·60	33	3275
1·65	21	3486
1·70	10	3520
1·75	3	3817
1·80	1	3400
1·85	2	4125
Average vital capacity per square metre of body surface		2050

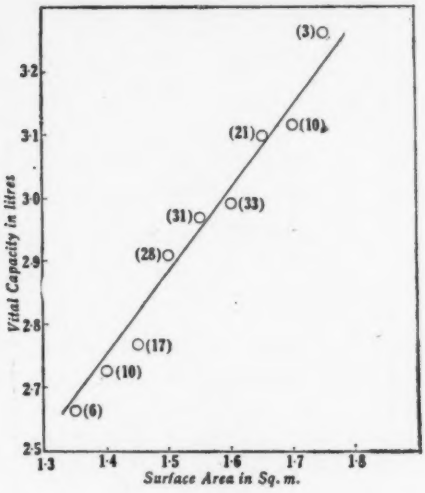

FIG. 6

TABLE VII

Average Vital Capacity according to Chest Expansion

Chest expansion in cm.	Number of persons	Average vital capacity in c.c.
5·60	27	2914
6·85	39	3165
8·10	47	3195
9·35	33	3339
10·60	13	3465

Figs. 3–7 represent the correlations that exist between vital capacity and the recorded measurements of the body. It is observed that whilst the vital capacity is nearly a linear function of standing height, stem height, surface area and chest expansion (Figs. 3, 5, 6 and 7), its relationship with weight (Fig. 4) is not so simple.

All the workers in the field accept that constant correlations exist between vital capacity and these measurements of the body but opinions are differed as to the relative importance of these correlations. Hutchinson[5] found the closest correlation between vital capacity and standing height.

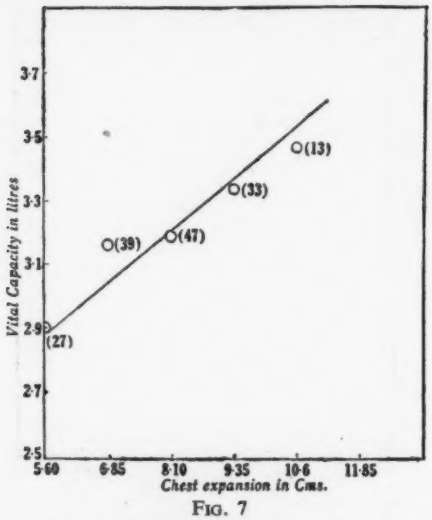

FIG. 7

West[5] observed the vital capacity to increase by 25 c.c. for addition of every centimetre in height. Boynton, Shepard, Maeder, Cady and Myers[5] have found "West's ratio to yield reasonably satisfactory results". On comparing standards based on different measurements of the body West advises the one based on surface area. That the vital capacity does not bear so close a correlation either with weight or stem height as it does with surface area, can be seen from the statements of Dreyer, West, Lemon, Boynton, Rogers and others.[5]

TABLE VIII

	Mean	Standard error of the mean	Standard deviation	Standard error of the standard deviation
Standing height in cm.	166·17	±0·5352	6·8510	±0·3764
Weight in Kg.	51·60	±0·4315	5·7236	±0·3035
Surface area in sq. m.	1·55	±0·0084	0·1078	±0·0059
Sitting height in cm.	85·51	±0·2695	3·4500	±0·1895
Chest expansion in cm.	7·65	±0·1248	1·5600	±0·0882
Vital capacity in litres	3·191	±0·0379	0·4856	±0·0267
Vital capacity per sq. m. of surface area	2·05	±0·0174	0·2250	±0·0124

TABLE IX

Correlation Coefficients

Height and Vital capacity ..	0·6380	±0·0460
Weight and Vital capacity ..	0·5019	±0·0576
Surface area and Vital capacity ..	0·5883	±0·0577
Sitting height and Vital capacity ..	0·5928	±0·0504
Chest expansion and Vital capacity	0·2863	±0·0834

The correlation coefficients of the present series given in Table IX, reveal that the vital capacity bears the highest correlation with standing height, slightly lower correlations with stem height and surface area but poor correlations with weight and chest expansion. Moreover, whilst the correlation co-efficients of stem height and surface area do not significantly differ from that of height, those of weight and chest expansion do significantly differ from it. It is generally recognised that "vital capacity is more nearly a function of body surface than of any other measurement" (Lemon and Moersch). A somewhat lower correlation between vital capacity and surface area, seen in Mysore students, is attributable to the low weight for a given height of these subjects. The average vital capacity of Mysore students represents widely differing percentages, such as 77, 89 and 82 per cents. of the normal for an American based on height, weight and surface area standards respectively, showing of all the recorded measurements of the body of Mysore students, the weight is relatively the lowest. It is also lower than that of either Bombay or Madras students. This can in part be attributed to a comparatively low effective nutrition.

It becomes obvious, from the above discussion, that for Mysoreans vital capacity standards based on standing height, stem height and surface area have better application than those based on any other measurement of the body. To calculate easily the average vital capacity for an adult Mysorean, regression equations of vital capacity based on height, stem height and surface area, are derived (from the data of Tables III, V and VI) and they are as follows:—

Vital capacity in litres = 0·0452 standing height in cm. −4·299

" " " = 0·0834 stem height in cm. −3·908

" " " = 2·651 surface area in sq.m. −0·891

TABLE X

A Comparative Table of Results of Vital Capacity Measurements obtained in Different Places in India

Authors and place	Number of subjects	Average height in cm.	Average weight in Kg.	Average surface area in sq.m. (S.A.)	Average vital capacity in litres	Average vital capacity in litres per sq.m. of S.A.	Average vital capacity per cm. of height in c.c.
Bhatia (Bombay)	100	166·9	53·0	1·577	3·091	1·96	18·52
Krishnan and Vareed (Madras)	103	166·4	52·6	1·580	2·930	1·85	17·50
	198	165·0	53·9	1·590	3·050	1·93	18·50
Mukherjee and Gupta (Calcutta)	12	168·0	52·4	1·580	3·560	2·225	21·20
Hiriyannaiya (Mysore) ..	166	166·1	51·6	1·550	3·191	2·05	19·10

Discussion

The average vital capacity of the Mysore students is 3·191 litres and 19·1 c.c. per cm. of height. It is much lower than the average for the American students (4383 c.c. as noted by Jackson)[6] and is only 77 per cent. of the average for Americans of the same height as the mean height of Mysore students. Not only in students of Mysore but also in those of Madras and Bombay (Table X) the vital capacity is lower than that of the Americans both absolutely and relatively. The nature of the diet, exercise, climate and racial characteristics must be reckoned with in any consideration of the factors that contribute to the reduction in vital capacity. The lower vital capacity values found in Oriental races of people living in tropics have invariably been attributed by the earlier workers (Dreyer, Edwards and Wilson, Hsieh, Myers[5] and Bhatia[1]) to the influence of "racial factor". Myers and Sheppard have shown that "the vital capacity is decreased 10 per cent. or more in cases of underweight". In health the common cause of low weight is want of proper nutrition. The body weight for a given height of Indians [Bombay, Madras, Calcutta and Mysore (Table X)] is lower than that of Americans of the same height. This factor alone partly contributes to the lower vital capacity values observed in Indians. Moreover, that the tropical climate adversely influences the vital capacity has been made out by Turner and Aboushadid[7] who found a reduction of 7·1 per cent. in the vital capacity of a group of English, American and German women that had lived in Syria from 2–10 years. These authors observed a reduction of 12·1 per cent. in the vital capacity of Syrian women

which they attributed to the 'racial factor'. The result of this isolated observation regarding the reduction in the vital capacity in Syrian women leading a quiet life without any manual labour can hardly be of general application and indeed has no logical force. The influence, if any, of the racial factor in lowering the vital capacity of people inhabiting the tropics can only be determined by an extensive study. It seems to me that the available information on this question is insufficient to attribute the lower vital capacity in the Orientals to a racial factor.

Whether climatic conditions (due to variations in altitudes and distances from the sea) of different places in tropics affect the vital capacity has not been so far clearly made out. The vital capacity of Bombay and Madras students (Table X) is lower by 100–150 c.c. in absolute quantity, *i.e.*, by 0·6 per cm. of height and by 0·09–0·12 litres per sq. metre of surface area, than that of Mysore students. The difference amounting to about 3–6 per cent. (according to the different standards used) is no doubt small. But the fact to be noticed is whereas all the group of Mysore medical students whose vital capacity measurements are recorded here consists of people that have lived in altitudes of 2800–3300 ft. above sea-level, the group of Madras and Bombay medical students presumably comprise subjects of not only sea coast towns but also of inland places of varying altitudes. Whether the observed difference in the vital capacity becomes of real significance can only be judged when the data obtained on people that have lived for a sufficiently long time in places at sea-level become available.

Summary

1. The result of the study of vital capacity of 166 Mysore medical students is recorded.

2. The average vital capacity of Mysore students is calculated to be $3{\cdot}190 \pm 0{\cdot}379$ litres, 19·1 c.c. per cm. of height and $2{\cdot}05 \pm 0{\cdot}0174$ litres per sq. metre of surface area of the body. The correlation between vital capacity and height is found to be slightly higher than that between vital capacity and surface area. Low weight of the individuals is suggested as a possible factor to account for such a correlation.

3. The average vital capacity and vital capacity values per cm. of height and per sq. metre of surface area are all decidedly lower than those of Americans.

4. The factors that contribute to reduce the vital capacity of people living in tropics are considered.

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THE GROWTH OF THE SUGARCANE PLANT IN INDIA*

Part I. Age-Fertiliser Effects on the Physiology and Chemistry of Sugarcane

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1. Introduction

In crop production the two principal biological considerations involved are—what variety to use and how to grow it. These necessarily link agriculture with plant breeding on the one hand, and plant physiology on the other. Crop production is thus essentially a process of biological adjustment between heredity and environment, between the natural forces working in the soil and the atmospheric variables; and our effort to know them and to control them economically are most likely to increase the output and quality of produce.[59] For the realisation of the full potentialities of the improved strain, more homogeneous in form and function, it must be grown under conditions optimum for its growth. This can most effectively be achieved when a scientific analysis has been made of the nature of the interactions between the several edaphic and the climatic factors with special reference to the plant material under consideration, and of the functions that the different organs of the plant inherently perform to increase the output of matter. In considering such a rôle of complimentary factors, operating simultaneously or otherwise, attention is more closely drawn to such conditions as are liable to be controlled economically and to better ends. Among these, fertilisers both organic and inorganic rank high.

In spite of the fact that India produces the largest acreage of world's sugarcane, it has a relatively low yield both as regards its tonnage and sugar out-turn; there is clearly a great deal to be learned about the growth of the sugarcane plant in India grown under conditions of wide variability

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of edaphic and climatic factors over her vast expanse. Eliminating the effect of climate, in our own experiments at Benares, for instance, the yields of sugarcane in a given year have varied from 600–1200 mds. per acre in the manurial field experiments that have been in progress at this Research Institute for several years; the average yield on cultivator's farm is too low. Our knowledge of these is still largely traditional and no longer suited to the modern trend of scientific agriculture. The secret of the problem lies in discovering not merely the extent to which treatments affect yields and totals consisting of both effective and ineffective units but how in terms of component growth factors this is brought about, and in discovering any weak link in the chain of physiological processes accompanying the age-cycle of the cane. It would be probable that such a link might act as a limiting factor if the external conditions were such as to attenuate it.

Age and longevity, though factors of fundamental interest in the growth, development and yield of an organism, have become recognised as such only recently.[6,30,34,35,40,48–54,57] Dealing with considerations of growth mechanism in relation to the life duration of the organism and the several related plant activities, the summarised results of author's experimental studies into the physiology and chemistry of plant material extending over a period of twelve years were published in *Science and Culture*[57] in its Indian Science Congress Jubilee number in 1938. Summarising the evidences it was concluded that growth is an integrated summation of several correlated physiological activities, and its dynamics is conditioned essentially by the rapidity of assimilation, respiration, the state of colloidal hydration of the protoplasm, the nature of food substratum, permeability of protoplasm and translocation of materials, the mass of the reactive substratum, and the rate of metabolism of the protoplasm in the sense of the capacity for initial, final and average energy output as also the gradient of decline which the individual exhibits during its life-cycle and for which the momentum is supplied by the potentialities of the uniting germ cells of the organism.

The effect of added manures and fertilisers would naturally be expected to vitally affect the physiological state of the cells and thereby the dynamics of growth and development. The problem as to how much the growth efficiency of a crop is increased by adjusting the factor intensity must necessarily take into account the contribution that each individual factor makes to the growth efficiency of the organism.

Working on the deficiency of nutrient salts, Briggs[5] noted that deficiency of any of the essential elements lowers the assimilation rate. Considerable interest has since then been taken in investigating

the rôle of manurial ingredients on important functional activities of the plants.[1–4,7–29,31,32,36–39,55,58,60–63] Gregory and collaborators[11–13] working with barley noted that nitrogen deficiency had little effect upon carbon dioxide intake by the leaves but had a slightly depressing effect upon the rate of CO_2 output. Karl Hamner[14] working with tomato and wheat observed a marked increase in respiration rate by the application of nitrates provided an initial carbohydrate reserve was present, the rate being roughly proportional to the amount of carbohydrates.

Addition of phosphates as noted by Gregory and others[11,13] resulted in an increase in assimilation rate. Brenchley[4] investigated the influence of phosphates on barley and concluded that the importance of phosphates lies in the early stages of growth when its application is of vital importance. Lyon[24,25] experimenting with Elodea noted that phosphates promote respiration. Respiration rate of barley was observed by Gregory and Richards[11] to be subnormal under phosphorus deficiency, while Singh[44] noted that respiration was considerably augmented on the supply of phosphates.

The importance of potash has been recognised by many investigators.[2,9,12,15,21,31,32,37,56,60,62] Gregory and Richards[11] noted that respiration of barley in potash-deficient cultures was super-normal while assimilation was sub-normal. Richards[37] has further shown that as the level of external potassium concentration is lowered there is an increase in the assimilation rate. Nightingale and Robbins[31] reported an increase in total nitrogen under conditions of potash deficiency.

A correlation between nitrogen content and leaf colour was established as early as 1899 by Ville. Schertz[62] indicated that differences in chlorophyll content are caused by potash. Fertilisation with both phosphates and nitrates showed a decided increase in the amount of chlorophyll. Singh and others[58] noted that singly potash and phosphorus are more useful than nitrogenous nutrients in increasing the photosynthetic efficiency of leaves.

The influence of manurial treatments on the chemical composition of the plant or its individual parts has been the subject of investigation and valuable contributions have been made by a large number of investigators.[1,2,8,9,11–13,15,16,18,27–29,31,32,39] To cite but a few instances, McGinnis[28] attributes the differences in the protein content of wheat to the available nitrogen in the soil. Sanyal[61] found that superphosphate has the greatest influence on sugarcane in producing juice of highest purity and sucrose content. Potash fertilisation also leads to the maintenance of high purity whereas nitrogenous treatment gives the lowest values for sucrose

and highest for glucose. Increase in application of nitrogen was noted by Das[8] to result in a lowering of sucrose percentage. According to Noel Deer[33] phosphates have a beneficial effect on the sucrose percentage of sugarcane while potash has the reverse effect.

Working on the problem of maturation and ripening in sugarcane, Singh and Mathur[55] held that maturity is a physiological condition of the cane stem and a mere estimation of sucrose, purity, °Brix, reducing sugars, sucrose ratio, severally or jointly, is not likely to give a correct picture unless supplemented by estimations of respiration intensity, certain enzymic systems, internal CO_2 and O_2 concentrations and percentage of chemical composition of sugarcane, for, ripening in plant organs is conditioned by (i) the availability of higher carbohydrates, (ii) the rate at which these are broken down to simpler ones, (iii) rate of tissue respiration, and (iv) the ease with which the gaseous waste products are removed from, and oxygen supplied to, the interior tissues.

The literature reviewed above indicates that manurial ingredients have a profound influence upon the functional activities of the plant and its chemical composition. A clearer understanding of the effect of nutritive ingredients on the various aspects of plant metabolism would be necessary to a coherent picture of the physiology of sugarcane for providing the basis for field experiments and utility of particular manure in increasing the yield of crop plants. To the elucidation of some of these problems the series of researches conducted in this direction are presented in the following pages; the remainder, it is proposed to describe in successive series.

II. Experimentation

Pot culture technique.—Sugarcane (Co. 312) was grown in pots internally coated with a wax-mixture, and filled with well sieved and thoroughly mixed farm soil. To ensure proper germination the soil was damped before filling, and requisite quantity of water added before sowing. Cane setts of uniform size were germinated first in sand moistened with water; germinated setts of equal vigour were subsequently selected and planted one in each pot. The pots were uniformly arranged in randomised blocks, and fertilisers added in quantities previously determined by the treatments per pot individually. Only standard fertilisers such as ammonium sulphate (20·6% N), superphosphate (40·2% P_2O_5) and sulphate of potash (48·0% K_2O) were used to supply the three nutritive ingredients N, P and K respectively. The experiment was conducted for a period of two years. The details of the lay-out are as under:—

Lay-out.—

SYSTEM OF REPLICATION	..	randomised blocks of eight treatments each.
TREATMENTS	..	(i) Control with soil containing no fertiliser (C), (ii) Soil + 150 lbs. N per acre (N), (iii) Soil + 75 lbs. P_2O_5 per acre (P), (iv) Soil + 75 lbs. K_2O per acre (K), (v) Soil + 150 lbs. N and 75 lbs. P_2O_5 per acre (NP), (vi) Soil + 150 lbs. N and 75 lbs. K_2O per acre (NK), (vii) Soil + 75 lbs. P_2O_5 and 75 lbs. K_2O per acre (PK) and, (viii) Soil + 150 lbs. N, 75 lbs. P_2O_5 and 75 lbs. K_2O per acre (NPK). The amount of fertiliser added per pot was calculated on top-surface area basis of the pots and supplied to each pot separately.
MEDIUM OF GROWTH	..	Well sieved farm soil (sandy loam).
TIME OF APPLICATION	..	Soon after germination.
DATES OF SOWING	..	15th March 1937; 20th March 1938.
DATES OF HARVESTING	..	20th December 1937; 21st November 1938.

Care was taken to see that requisite quantity of water was uniformly supplied to all the pots. Hoeing and weeding was done in alternate weeks. The entire series of pots meant for sampling plant material for the determination of dry weight, photosynthetic efficiency, chlorophyll content, etc., were maintained separate from those reserved for studies on yield, composition of juice and other growth characters. This procedure was strictly followed so as to ensure that final yield and other determinations were not vitiated by any biased as selection of plants for other studies.

Characters studied and Methods used.—The characters studied consisted, among others, of growth measured by plant height, tillering, number of leaves on the main stem, length and breadth of leaf, number of exposed internodes on the stem, girth of stripped cane, dry weight accumulation, photosynthesis, chlorophyll content, respiration, and chemical estimations of

juice characters such as sucrose, glucose, °Brix and purity at successive stages of the life-cycle of cane.

The rates of photosynthesis and respiration were measured by the continuous current method after Blackman, F. F., under optimum conditions of external illumination, temperature and carbondioxide concentration keeping the cardinal values at those determined in an earlier contribution from these laboratories.[53] The determinations were made of leaf of the same age at successive stages. Bariumhydroxide of a known strength was used as an absorbent of carbondioxide, the value of CO_2 taken in or given out being determined by titrating against standard hydrochloric acid.

For chlorophyll estimation 1–2 gms. of the green leaf material (less mid-rib) was chopped into fine pieces and macerated with 5 c.c. of acetone-ether mixture (7 : 3). The extract along with the macerated tissue was then poured in a stoppered glass vessel and the volume made upto 25 c.c. by adding the ether-acetone mixture; the stoppered cell was kept away from light in constant temperature incubator at 30° C. for a period of 12–18 hours when the extraction was complete. The extinction measurements of the acetone-ether extract were measured by Singh and Rao's Photo-conductive Photo-meter[56]; the concentration of chlorophyll in the extract was then read from a calibration graph previously prepared with pure chlorophyll.

In all measurements where the study of leaf was deemed necessary, the third leaf from the top of the primary shoot was selected for the study of photosynthetic efficiency and chlorophyll content. This method of sampling was found to be fairly accurate in all comparative responses to added fertilisers. From the point of view of development also the leaves were found to be appropriate being morphologically well developed and usually free from diseased spots or malformations.

For dry weight measurements the plants were sampled out the previous evening. After carefully removing the superficial moisture, they were dried to constant weight in an air-oven maintained at a temperature of 105° C. For juice analysis composite samples from several bushes of similar treatments were used. Extraction percentage in three-roller mill was determined by finding out the quantity of juice per 100 gms. fresh weight. °Brix was determined by °Brix hydrometer, sucrose by means of Schmidt and Hans saccharometer using dryweight method, and glucose by Fehling's solution method. The purity coefficient of juice was derived from the values of sucrose and °Brix. Temperature corrections for °Brix determinations, and °Brix corrections for sucrose estimations were introduced after the methods indicated in the *Hand Book of Sugarcane Manufacturers and their Chemists.*

III. Experimental Findings

Growth in height.—Growth in height of sugarcane plants is found to vary in response to the individual fertilisers or a mixture thereof; the extent of variation from treatment to treatment assumes prominence as age advances. Thus during the early growth stage of cane (month of May) when the canes are 65 days old there is hardly any noticeable difference over the control on the application of N, NP, NK and NPK. In July (canes 120 days old) the measurable responses are but slight; in August (canes 170 days old) the responses become more prominent; specially is this true of NK-treated canes where the response is significantly obvious. Towards the end of the life-cycle, however, P, NP, NK, NPK-treated canes add distinctly to the height of the cane in the order of priority above given; this holds good for both the years, *viz.*, 1937–38 and 1938–39. The nature of general response in the two years remains similar inasmuch as while both nitrogen and phosphorus tend to increase height, potash markedly diminishes it. The increases due to nitrogen alone are significant in both the years (Table I).

Tillering.—Increase in the number of tillers per bush of cane for each of the added fertiliser is highest in July (canes 120 days old), *viz.*, during rains, subsequent to which a fairly large number of these perish. This is more marked in NPK and N-treated canes where the number of tillers is reduced from 10·3 and 10·0 to 4·0 and 3·1 respectively. The capacity for tillering responds similarly for other treatments as well. The nature of general responses indicates that sulphate of potash and ammonium sulphate have a tendency to increase tillering; that for added superphosphate to decrease it. The increases or decreases in the two years due to these are insignificant [Table I (1)].

Number of leaves.—The capacity for increase in the number of leaves reaches its uppermost limit in the month of July, *viz.*, during the middle of rains, when the canes are 120 days in age, after which it decreases. Nitrogen and potash tend to increase the number of leaves; phosphorus on the contrary retards it. The decreases due to phosphorus alone are significant in 1937–38, while the increases due to nitrogen and decreases due to phosphorus are both significant in 1938–39 [Table I (2)].

Leaf size.—The average responses of nitrogen, phosphorus and potash upon the length of leaves for the entire life-cycle are portrayed in Tables I (3) and I (4). Ammonium sulphate and sulphate of potash both tend to increase leaf length; phosphates on the other hand depresses leaf elongation. The increases and decreases are, however, not significant. Nitrogen, phosphorus and potash all tend to add to the breadth of the leaf; none of these give a significant response.

TABLE I

Mean height of sugarcane plant, 1937–38 *and* 1938–39

Date	Age in days	C	N	P	K	NP	NK	PK	NPK	Mean	General responses
20—5—1937 ..	65	2′ 4″	2′ 4″	2′ 3″	1′ 11·5″	1′ 11″	2′ 4″	2′ 6″	2′ 4″	2′ 3″	
13—7—1937 ..	120	5′ 3″	5′ 6″	5′ 6″	5′ 4″	5′ 8·5″	5′ 6″	2′ 6″	5′ 4″	5′ 1″	
30—8—1937 ..	170	7′ 7″	8′ 2″	8′ 11″	8′ 5″	8′ 10″	9′ 1·5″	8′ 6″	8′ 1″	8′ 5·4″	N = +5·75 ±1·09** P = +2·425 ±3·26 K = −1·225 ±3·19
1–10—1937 ..	215	8′ 0″	8′ 11″	9′ 2″	8′ 8″	9′ 10″	9′ 3″	9′ 2″	8′ 9″	8′ 11·6″	
3–11—1937 ..	250	8′ 0″	9′ 8″	10′ 2″	9′ 2″	10′ 8″	10′ 2″	9′ 5″	10′ 2″	9′ 8″	
Mean ..	..	6′ 2·8″	6′ 11″	7′ 2·4″	6′ 8·5″	7′ 4·7″	7′ 3·3″	6′ 5″	6′ 11·2″	..	
		cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	
5—5—1938 ..	36	42·3	51·0	31·33	50·33	48·23	53·46	34·33	51·66	45·33	
20—6—1938 ..	62	79·33	113·0	96·00	131·33	119·00	141·66	89·33	133·66	112·91	N = +44·115 ±14·27** P = + 1·4 ±18·61 K = − 3·64 ±16·46
20—9—1938 ..	183	199·66	243·33	226·66	236·66	255·66	263·33	201·66	274·33	237·66	
Mean ..	..	107·09	135·77	117·99	139·44	207·63	152·81	108·44	153·21	..	

** 1% Significance

TABLE I (1)

Mean number of tillers per plant

Date	Age in days	C	N	P	K	NP	NK	PK	NPK	Mean	General responses
20—5—1937 ..	65	4·30	4·7	5·5	4·8	3·8	4·8	4·7	4·3	4·6	
13—7—1937 ..	120	8·00	9·3	10·0	8·3	8·0	9·3	8·18	10·3	9·0	
30—8—1937 ..	170	6·5	6·3	5·5	6·5	6·0	6·0	6·1	5·7	6·1	N = −0·08 ±0·19 P = −0·14 ±0·18 K = +0·01 ±0·18
1–10—1937 ..	215	3·8	4·1	4·0	4·0	3·5	3·1	2·3	3·0	3·5	
3–11—1937 ..	250	4·0	3·5	3·1	4·1	3·5	3·5	4·0	4·0	3·7	
Mean ..	..	5·32	5·58	5·62	5·54	4·96	5·34	5·18	5·46	..	
5—5—1938 ..	36	3·1	4·5	2·3	7·4	4·2	6·8	2·0	6·3	4·5	
20—6—1938 ..	62	2·9	4·2	3·8	6·3	3·4	6·1	2·2	5·6	4·3	N = +1·2 ±0·80 P = −1·18 ±0·98 K = +1·22 ±0·93
20—9—1938 ..	183	2·3	5·9	4·7	6·3	4·1	4·2	2·4	4·7	4·3	
Mean ..	..	2·76	4·86	3·6	6·6	3·9	5·7	2·2	5·5	..	

TABLE I (2)

Number of leaves on the main shoot

Date	Age in days	C	N	P	K	NP	NK	PK	NPK	Mean	General responses
20—5—1937 ..	65	8·0	8·5	7·6	7·1	7·3	7·6	8·0	8·0	7·6	
13—7—1937 ..	120	13·0	12·1	12·0	12·0	11·3	12·6	12·0	12·0	12·1	
30—8—1937 ..	170	11·8	11·1	11·6	11·1	10·3	11·5	11·5	11·3	11·2	N = +0·225 ±0·287** P = −0·435 ±0·12** K = +0·195 ±0·28
1–10—1937 ..	215	9·6	10·0	10·8	9·8	10·0	12·0	8·1	11·5	10·2	
3–11—1937 ..	250	9·0	9·8	8·0	9·5	8·0	10·0	9·0	9·1	9·0	
Mean ..	..	10·28	10·3	10·0	9·9	9·38	10·74	9·72	10·38	..	
5—5—1938 ..	446	10·1	12·6	10·2	10·1	10·0	11·3	8·1	12·2	10·5	
20—6—1938 ..	92	10·1	12·4	9·4	11·2	11·1	12·2	8·0	11·1	10·6	N = +1·47 ±0·36** P = −1·02 ±0·28** K = +0·07 ±0·36
20—9—1938 ..	183	9·1	8·0	8·0	8·8	8·7	10·4	8·3	9·0	8·7	
Mean ..	..	9·76	11·0	9·2	10·03	9·93	11·3	8·1	10·76	..	

** 1% Significance

TABLE I (3)

Length of the longest leaf on the main shoot

Date	Age in days	C	N	P	K	NP	NK	PK	NPK	Mean	General responses
20—5—1937 ..	65	1′ 11·5″	1′ 10″	1′ 9″	1′ 7″	1′ 7″	1′ 11″	1′ 11″	1′ 11·5″	1′ 10″	
13—7—1937 ..	120	3′ 3·0″	3′ 0″	3′ 2·5″	2′ 11″	3′ 2″	3′ 11″	3′ 2″	2′ 11″	3′ 5·1″	
30—8—1937 ..	170	3′ 9·5″	4′ 1″	4′ 3″	4′ 2″	4′ 3″	4′ 6″	4′ 3″	3′ 9″	4′ 2·1″	N = +1·62 ± 1·17 P = −0·27 ± 1·22 K = +0·67 ± 1·20
1–10—1937 ..	215	3′ 4″	3′ 11·5″	3′ 11″	3′ 11·5″	3′ 10″	4′ 5″	3′ 8″	4′ 6″	3′ 11·4″	
3–11—1937 ..	250	3′ 2″	3′ 5″	3′ 8″	3′ 3″	3′ 7·5″	3′ 5″	3′ 9″	3′ 3″	3′ 5·3″	
Mean ..	..	3′ 2″	3′ 3·1″	3′ 4·3″	3′ 2·1″	3′ 3·5″	3′ 7·6″	3′ 2·6″	3′ 3·3″	..	

TABLE I (4)

Breadth of the longest leaf on the main shoot (inches)

Date	Age in days	C	N	P	K	NP	NK	PK	NPK	Mean	General responses
20—5—1937 ..	65	0·71	0·6	0·5	0·56	0·57	0·65	0·66	0·71	0·62	
13—7—1937 ..	120	0·95	0·6	0·5	0·93	0·90	0·86	0·96	0·78	0·81	
30—8—1937 ..	170	0·96	1·01	1·13	1·08	1·23	1·25	1·1	1·5	1·02	N = +0·04 ± 0·65 P = +0·09 ± 0·08 K = +0·01 ± 0·05
1–10—1937 ..	215	1·0	1·08	1·25	1·15	1·73	1·2	1·2	1·16	1·20	
3–11—1937 ..	250	1·0	1·01	1·16	1·1	1·4	1·0	1·2	0·8	1·09	
Mean ..	..	0·92	0·86	0·91	0·96	1·18	0·97	1·02	0·99	..	

TABLE I (5)

Number of internodes exposed on the main shoot

Date	Age in days	C	N	P	K	NP	NK	PK	NPK	Mean	General responses
20—5—1937 ..	65	8·8	9·6	10·5	9·1	8·8	9·5	10·1	8·18	9·40	
13—7—1937 ..	120	8·8	10·8	10·8	9·1	8·8	9·1	10·0	9·8	9·65	
30—8—1937 ..	170	8·0	6·1	9·5	7·8	7·8	8·3	6·3	7·5	7·66	N = −0·24 ±0·37 P = +0·47 ±0·27 K = −0·265 ±0·33
1–10—1937 ..	215	8·6	8·5	10·1	8·3	8·8	9·5	8·0	9·0	8·82	
3–11—1937 ..	250	10·1	8·5	10·1	8·3	9·5	8·6	10·0	10·0	9·37	
Mean ..	..	8·84	8·7	10·2	8·52	8·74	9·0	8·88	9·02	..	

TABLE I (6)

Girth of the main shoot in inches

Date	Age in days	C	N	P	K	NP	NK	PK	NPK	Mean	General responses
20—5—1937 ..	65	1·1	1·1	1·1	1·0	1·1	1·2	1·2	1·2	1·11	
13—7—1937 ..	120	2·8	2·7	2·4	2·4	2·5	2·4	2·5	2·4	2·51	
30—8—1937 ..	170	2·8	2·8	2·5	2·4	2·5	2·7	2·5	2·5	2·76	N = −0·05 ±0·018* P = −0·11 ±0·018** K = +0·03 ±0·02
1–10—1937 ..	215	2·2	2·4	2·5	2·5	2·44	2·8	2·5	2·5	2·47	
3–11—1937 ..	250	2·9	2·5	2·5	2·5	2·5	2·5	2·8	2·5	2·57	
Mean ..	..	2·36	2·3	2·2	2·36	2·2	2·32	2·3	2·2	..	

* 5% Significance ** 1% Significance

Number of exposed internodes.—There is absolutely no significant response to any one of the three nutriants with respect to this character, although there exists a tendency of nitrogen and potash to decrease the number of nodes exposed, and of phosphorus to increase it [Table I (5)].

Girth of cane stem.—Added ammonium sulphate and phosphates significantly decrease cane girth, but increase height. Sulphate of potash shows significant increases in girth at the cost of height [Table I (6)].

Dry matter accumulation.—The absolute dry weight of entire plant continues to increase steadily under all the treatments. During the early stages of growth the differences in dry weight per plant are not clearly marked amongst the treatments themselves, but with advance in age wider differences become noticeable from treatment to treatment. The responses of nitrogen, phosphorus or potassic fertilisers in the first year of experiment are thus not significant for the major earlier portion of the life-cycle; there is all the same a distinct tendency on the part of nitrogen to increase dry matter production. Only towards the end of the age-cycle of cane does the response due to nitrogen become positively significant. In the second year of experimentation, however, nitrogen gives highly significant responses in increasing dry matter accumulation at all the stages of life-cycle; phosphorus and potash show significant responses only once in the life-cycle, when the canes are 114 days in age. The order in which the final dry weights of the differently treated plants stand are:

1937–38 .. NP > NK > N > PK > NPK > P > K > C

1938–39 .. NP > NK > N > NPK > P > PK > K > C

Canes supplied with a mixture of phosphates and nitrogen consistently show maximum dry weight production in both the years; clearly thus nitrogen applied singly or in combination makes for superior response in dry matter accumulation while treatments lacking in nitrogen produce inferior quantity of dry matter (Table II).

Mean apparent assimilation.—The rate of apparent assimilation in sugarcane leaves exhibits a general tendency to augment in response to the application of all the fertilisers as judged by the general positive responses of each of these (Table III) at a stage when the canes are 212 days in age. In 1938–39 the response at successive stages differs slightly: nitrogen alone increases assimilation during the entire life-cycle; increase during the later stage alone being significant. The effects of phosphates and potash are only negative and insignificant all through the age-cycle of the cane.

TABLE II

Dry weight of entire plant at successive stages of life-cycle

Date	Age in days	C	N	P	K	NP	NK	PK	NPK	Mean	General responses
20—5—1937	65	22·45	25·74	18·28	16·37	23·96	21·45	21·79	25·35	20·67	N = + 1·90 ± 2·51 P = + 1·66 ± 1·82 K = + 1·13 ± 2·22
13—7—1937 ..	120	168·98	132·62	145·44	153·95	166·41	225·95	133·15	117·49	155·5	N = + 10·24 ± 20·57 P = − 29·75 ± 25·42 K = + 4·27 ± 26·77
30—8—1937 ..	170	324·1	246·3	308·4	331·9	468·2	451·9	353·6	291·9	359·5	N = + 60·09 ± 43·2 P = − 7·79 ± 50·4 K = − 4·42 ± 52·6
1—10—1937 ..	215	373·9	421·6	338·3	364·4	574·7	484·7	385·7	309·5	406·6	N = + 82·06 ± 56·7 P = − 9·11 ± 58·9 K = − 41·05 ± 66·1
21-11—1937 ..	268	380·9	515·0	442·7	433·4	601·5	574·7	466·7	462·3	484·6	N = +107·4 ±46·75** P = + 17·3 ±38·6 K = − 0·75 ±40·7
25—5—1938 ..	66	18·31	22·41	19·32	17·49	26·96	24·27	19·85	22·42	21·37	N = + 5·27 ± 1·02** P = + 1·52 ± 1·21 K = − 0·74 ± 1·11
12—7—1938 ..	114	115·3	129·2	136·9	160·0	180·2	195·7	207·3	218·4	167·8	N = + 26·0 ± 6·90** P = + 35·65 ± 4·78** K = + 54·95 ± 6·88**
18—8—1938 ..	151	180·3	325·3	301·4	317·1	419·9	440·2	309·2	339·8	329·1	N = +104·3 ±42·32* P = + 24·35 ±45·78 K = + 44·85 ±43·55
6—10—1938 ..	200	340·9	450·4	359·2	401·9	605·2	670·8	456·4	498·3	472·8	N = +166·6 ±47·14** P = + 13·8 ±59·3 K = + 67·9 ±52·02
16-11—1938 ..	241	432·9	672·2	598·6	505·7	720·3	695·9	531·4	660·9	602·2	N = +170·7 ±64·34* P = + 51·3 ±36·29 K = − 7·75 ±29·18

* 5% Significance ** 1% Significance

* 5% Significance ** 1% Significance

TABLE III

Mean apparent assimilation rate of leaves (1937–38; 1938–39)

Age in days	C	N	P	K	NP	NK	PK	NPK	Mean	General responses
1937–38										
212	2·171	2·567	0·545	3·854	4·741	3·156	3·322	3·450	2·975	N = +1·005 ±0·93 P = +0·343 ±0·67 K = +0·94 ±0·75
1938–39										
45 ..	8·72	30·35	23·89	27·03	− 4·53	21·21	4·64	18·56	16·23	
90 ..	12·09	20·90	4·80	11·34	50·83	1·56	6·38	9·20	14·64	N = +2·81 ±3·62 P = −1·47 ±2·85 K = −6·875 ±3·77
135 ..	5·77	14·27	15·00	8·02	4·16	1·12	9·01	2·78	7·51	
Mean 45–135 ..	8·86	21·84	14·56	15·46	16·82	7·96	6·68	10·18	..	
180 ..	4·95	16·29	7·78	12·18	2·74	9·68	−11·47	11·59	6·72	
225 ..	12·80	23·11	21·14	20·26	21·62	26·51	15·47	20·86	20·22	N = +4·27 ±1·63* P = −2·30 ±1·28 K = −1·33 ±2·05
260 ..	− 5·54	− 6·90	− 4·00	−11·90	− 2·17	− 2·81	− 3·50	−10·04	− 5·98	
Mean 180–260 ..	4·07	10·83	8·31	6·85	7·39	10·79	0·16	7·47	..	

* 5% Significance

TABLE III (1)

Mean respiration of sugarcane leaves (1937–38; 1938–39)

Age in days	C	N	P	K	NP	NK	PK	NPK	Mean	General responses
1937–38										
212	3·876	5·455	7·816	6·209	6·376	6·115	7·013	5·934	6·099	N = −0·283 = ±0·58 P = +1·021 ±0·57 K = +0·437 ±0·61
1938–39										
45 ..	8·72	1·59	8·13	10·25	10·21	28·69	9·28	6·02	10·36	
90 ..	7·15	23·10	9·60	13·61	8·62	16·45	17·89	13·10	13·69	N = +4·42 ±1·21** P = −1·85 ±0·79* K = +6·84 ±1·03**
135 ..	2·88	4·34	0·08	10·08	3·70	13·39	5·00	26·47	8·24	
Mean 45–135 ..	6·25	9·67	5·93	11·31	7·51	19·51	10·72	15·19	..	
180 ..	2·61	3·21	3·33	9·00	28·78	1·65	17·01	7·11	9·08	
225 ..	2·40	13·86	4·00	13·25	10·51	3·50	3·38	13·41	8·04	N = +2·865 ±2·83 P = +3·54 ±1·72* K = +0·65 ±2·27
260 ..	1·20	1·20	0·90	1·10	1·23	2·87	1·76	7·01	2·16	
Mean 180–260 ..	2·07	6·09	2·74	7·78	13·50	2·67	7·38	9·17	..	

* 5% Significance ** 1% Significance

TABLE III (2)

Mean real assimilation rate of sugarcane leaves (1937–38; 1938–39)

Age in days	C	N	P	K	NP	NK	PK	NPK	Mean	General responses
1937–38										
212	6·047	8·022	7·271	10·063	11·117	9·271	10·335	9·384	8·938	N = +1·119 ±0·96 P = +1·126 ±0·55* K = +2·51 ±0·55**
1938–39										
45 ..	17·44	31·94	32·02	37·28	5·68	49·90	13·94	24·58	26·59	N = +7·14** P = −3·39** K = +1·47*
90 ..	19·24	44·00	14·40	24·95	59·45	18·01	24·27	22·30	28·32	NP = −1·24* NK = −2·50**
135 ..	8·64	18·61	15·08	19·10	7·86	14·52	14·01	29·25	15·88	PK = −2·97** NPK = +5·05**
Mean 45–135 ..	15·1	31·51	20·5	27·11	24·33	27·47	17·40	25·37	..	S.E. = ±0·677
180 ..	7·56	19·50	11·11	21·18	31·52	11·33	5·54	18·70	17·05	N = +7·07** P = −1·17** K = −0·75*
225 ..	15·20	36·97	25·14	33·51	32·13	30·01	18·85	34·27	28·26	NP = +2·4** NK = −3·13**
260	− 4·34	− 5·70	− 3·10	−10·8	− 0·896	− 0·96	− 1·74	− 2·77	− 3·80	PK = −3·13** NPK = +2·73**
Mean 180–260 ..	6·42	16·92	11·05	14·63	20·90	13·46	7·5	16·64	..	S.E. = ±0·288

* 5% Significance ** 1% Significance

TABLE III (3)

Mean chlorophyll content of sugarcane leaves (1937–38; 1938–39)

Age in days	C	N	P	K	NP	NK	PK	NPK	Mean	General responses
1937–38	18·0	21·9	22·5	22·5	25·1	23·5	21·7	21·7	22·0	N = +1·62 ±0·88 P = +1·27 ±1·32 K = +0·475 ±1·46
1938–39										
45 ..	4·4	8·2	7·5	6·5	6·9	5·1	6·4	8·5	6·7	
135 ..	5·1	9·3	7·5	6·4	6·2	6·1	6·4	9·0	6·9	N = +1·58 ±0·97 P = +0·76 ±1·06 K = −0·19 ±1·06
225 ..	6·1	10·1	7·5	6·3	7·8	7·1	5·8	10·5	7·65	
Mean ..	5·20	9·20	7·46	6·40	6·96	6·10	6·20	9·33	..	

Mean respiration rate.—Both phosphorus and potash show positive responses in so far as the rates of respiration are concerned, and nitrogen, a negative one. The increases or decreases due to these are insignificant during the experimentation of 1937–38 [Table III (1)]; in 1938–39, however, nitrogen and potash both significantly increase respiration while phosphates tend to depress it. As canes advance in age, the increase due to phosphates alone is significant. The general responses due to nitrogen and potash are positively significant. It would thus seem that phosphates have a depressing effect on respiration during the early stages of cane's development and a distinct augmentative effect on the respiratory activity during the later periods of growth; the age factor may thus have masked the effect of phosphates in the earlier part of the life-cycle.

Mean real assimilation.—During 1937–38, the mean responses to ammonium sulphate, superphosphates, and sulphate of potash are all positive but significant only in case of the latter two. Potash seems to have greater effect in augmenting rate of real assimilation than either phosphates or nitrogen. In 1938–39, for the first half of the life-cycle, the responses due to N, K and NPK are positively significant, while those due to P, NP, NK, and PK negatively so; during the second half (6–9 months) the responses are shown to be positively significant only in the case of N, NP and NPK, and for the rest negatively significant. All the main effects and interactions are significant [Table III (2)].

Chlorophyll content.—The chlorophyll content of each of the treated leaves is higher than the control consistently at all the stages in the life-cycle. The general responses due to nitrogen and phosphorus are positive though insignificant during the two years of experimentation; potash indicates a positive response in 1937–38, and a negative one in 1938–39. Both the increases and decreases remain, however, insignificant [Table III (3)].

Mean yield.—The order in which the yields of stripped cane are obtained in the two years stands thus:

1937–38 .. NK > NP > P > PK > K > NPK > N > C.

1938–39 .. NP > NK > P > K > NPK > PK > N > C.

All the main effects are positively significant in the first year; that due to nitrogen alone is so in the second. Potash significantly depresses the yield in the second year. The interactions are significant in one year and insignificant in another (Table IV).

TABLE IV

Mean yield of stripped cane (1937–38; 1938–39) *per pot in gms.*

Treatment	1937–38		1938–39	
	Mean Yield	Responses	Mean Yield	Responses
C	725·8	..	805·0	..
N	885·0	+ 96·12**	890·7	+234·23**
P	1008·7	+ 76·07**	1130·3	+ 48·68
K	954·2	+ 28·22**	1022·7	− 52·88*
NP	1130·0	− 71·37**	1140·3	+ 10·83
NK	1130·4	− 43·47**	1135·4	− 4·03
PK	966·0	−188·17**	978·3	− 43·68
NPK	894·0	− 52·52**	999·5	− 38·82
S.E.	..	± 3·29	..	± 26·16

* 5% Significance ** 1% Significance

Juice characters: *Sucrose*.—The sucrose content of the juice varies with the added fertilisers at all stages of cane development. Taking overall values at successive stages in 1937–38 the tendency of nitrogen is to diminish sucrose percentage, that of potash and phosphates to increase it. Potash alone significantly increases sucrose accumulation in the experiments of first year; during the second the tendency of potash and phosphates is more or less similar to that in the first year, while that of nitrogen gives a positive response. On the basis of the final values the responses arrange themselves thus:

1937–38 .. PK > NPK > K > N > NK > P > NP > C

1938–39 .. PK > K > C > P > NPK > NK > NP > N.

That mixture of phosphates and potash gives the highest percentage of sucrose is made evident consistently in both the years (Table V).

Glucose.—The response of glucose to fertilisers varies in the two years. Phosphates show a positive response in the first year and negative in the second: nitrogen and potash have negative responses in the first year and positive in the second. Whatever the nature of responses, these are insignificant but for potash which gives significant increase in 1938–39 [Table V (1)].

TABLE V

Sucrose content in sugarcane juice (1937–38; 1938–39)

Age in days	C	N	P	K	NP	NK	PK	NPK	Mean	General responses
1937–38										
177 ..	2·4	2·45	3·05	3·15	2·05	2·75	3·65	2·56	2·76	
201 ..	3·65	4·25	5·01	5·35	3·75	4·15	5·45	4·82	4·55	
215 ..	6·85	7·75	7·85	8·01	6·30	6·80	8·82	7·25	7·45	N = −0·225 ±0·30
235 ..	9·95	10·05	9·35	9·95	9·80	9·95	11·3	9·7	10·01	P = +0·086 ±0·35
259 ..	11·9	13·8	11·2	12·8	10·9	12·8	13·75	12·5	12·46	K = +0·631 ±0·28*
273 ..	13·65	15·1	14·7	15·6	14·6	15·0	16·25	16·15	15·13	
Mean ..	8·06	8·9	8·52	9·14	7·9	8·57	9·37	8·83	..	
1938–39										
130 ..	8·30	9·20	9·30	8·70	9·30	9·00	8·60	9·50	8·98	
172 ..	9·10	10·50	10·60	9·70	11·40	9·80	9·50	11·00	10·20	N = +0·18 ±0·48
209 ..	10·12	12·20	14·26	13·70	12·11	11·00	11·80	15·23	12·55	P = +0·67 ±0·49
240 ..	13·85	12·30	13·55	13·85	12·35	12·85	14·25	13·25	13·26	K = +0·13 ±0·48
Mean ..	10·34	11·05	11·92	11·48	11·29	10·66	10·76	12·24	..	

* 5% Significance

TABLE V (1)

Glucose percentage in sugarcane juice (1937–38; 1938–39)

Age in days	C	N	P	K	NP	NK	PK	NPK	Mean	General responses
1937–38										
177 ..	2·25	2·1	3·72	3·34	2·79	2·90	2·05	2·31	2·68	
201 ..	1·85	1·92	3·35	3·18	2·56	2·34	1·92	2·04	2·39	
215 ..	1·52	1·75	2·90	2·84	2·18	2·07	1·67	1·83	2·09	N = −0·272 ± 0·19 P = +0·049 ± 0·50 K = −0·005 ± 0·41
235 ..	1·25	1·35	2·25	2·19	1·64	1·92	1·33	1·53	1·68	
259 ..	1·01	1·12	1·95	1·72	1·23	1·32	1·00	1·10	1·30	
273 ..	0·96	0·86	1·67	1·66	1·00	1·10	0·80	0·90	1·12	
Mean ..	1·473	1·516	2·64	2·48	1·89	1·94	1·46	1·61	..	
1938–39										
130 ..	1·54	1·21	1·11	1·49	1·10	1·31	1·51	1·05	1·29	
172 ..	1·40	1·00	1·10	1·13	0·93	1·11	1·25	0·98	1·12	N = +0·009 ± 0·32 P = −0·037 ± 0·037 K = +0·080 ± 0·04*
209 ..	0·54	0·42	0·49	0·44	0·32	0·41	0·51	0·93	0·52	
240 ..	0·85	1·25	0·95	1·02	1·45	1·20	0·90	1·40	1·12	
Mean ..	1·07	0·97	0·91	1·02	0·95	1·07	1·04	1·09	..	

* 5% Significance

TABLE V (2)

Brix in sugarcane juice (1937–38; 1938–39)

Age in days	C	N	P	K	NP	NK	PK	NPK	Mean	General responses
1937–38										
177 ..	5·80	5·90	6·50	6·80	5·10	6·20	7·20	6·20	6·21	
201 ..	7·30	8·50	9·30	10·30	7·50	8·40	10·80	9·30	8·92	
215 ..	9·80	10·40	10·90	11·40	8·90	9·70	12·60	10·10	10·47	N = −0·47 ±0·51 P = +0·012 ±0·45
235 ..	12·60	13·50	12·30	12·60	12·20	12·70	13·30	12·00	12·65	K = +0·89 ±0·39*
259 ..	13·50	15·90	13·10	14·90	12·90	14·80	15·90	14·10	14·40	
273 ..	15·70	18·10	16·90	18·80	16·80	18·00	19·10	18·90	17·80	
Mean ..	10·78	12·05	11·50	12·46	10·56	11·63	13·15	11·76	..	
1938–39										
240 ..	17·2	17·0	17·0	16·8	16·2	16·5	17·3	17·0	..	N = −0·40 ±0·12** P = +0·0 ±0·27 K = +0·05 ±0·24

* 5% Significance ** 1% Significance

TABLE V (3)

Purity coefficient of sugarcane juice (1937–38; 1938–39)

Age in days	C	N	P	K	NP	NK	PK	NPK	Mean	General responses
1937–38										
177 ..	41·37	41·5	46·9	46·3	40·1	44·3	50·6	41·2	44·03	
201 ..	50·00	50·0	54·4	51·9	50·0	49·4	50·3	51·8	50·97	
215 ..	69·8	74·5	72·01	70·3	70·7	70·1	70·0	71·8	71·15	N = −1·08 ±0·27**
235 ..	78·9	74·4	76·0	78·9	80·3	78·3	84·9	80·8	79·06	P = +0·89 ±0·32*
259 ..	88·1	86·7	86·1	85·9	84·5	86·5	86·4	88·6	86·6	K = +0·83 ±0·37*
273 ..	86·9	83·4	86·9	82·9	86·9	83·3	85·4	85·4	85·14	
Mean ..	69·17	68·41	70·38	60·36	68·75	68·69	71·10	69·93	..	
1938–39										
240 ..	80·5	72·3	79·7	82·4	76·2	77·8	81·7	77·9	78·56	N = −5·07 ±0·96**
										P = +0·62 ±0·96
										K = +2·775 ±0·79**

TABLE V (4)

Extraction percentage of sugarcane (1937–38; 1938–39)

Age in days	C	N	P	K	NP	NK	PK	NPK	Mean	General responses
1937–38										
273 ..	51·9	52·3	51·6	52·3	53·7	56·55	54·8	53·5	53·32	N = +1·36 ±1·03
										P = +0·16 ±1·05
										K = +1·91 ±1·06
1938–39										
240 ..	55·8	61·5	60·4	59·2	64·6	64·0	59·2	65·6	61·28	N = +5·27 ±0·43**
										P = +2·32 ±0·86*
										K = +1·42 ±0·86

* 5% Significance ** 1% Significance

°*Brix and purity* of the juice respond almost similarly in the two years inasmuch as nitrogen tends to decrease them and phosphates and potash tend to increase these. Potash significantly increases °Brix during the first year while nitrogen significantly depresses it during the second year. The responses in other cases are not significant. Purity on the other hand is significantly increased in response to potash and significantly depressed in response to nitrogen in both the years. Phosphorus shows positive significance only during 1937–38 [Tables V (2) and V (3)].

The juice percentage shows tendency to increase due to the application of all the three ingredients. The increases due to nitrogen and phosphorus however, are significant during the second year of experiment [Table V (4)].

IV. Discussion

An analysis of the data obtained in the course of these investigations has revealed that the various cane characters studied respond markedly to added sulphate of ammonia, superphosphates and sulphate of potash; the degree of responses of the entities vary amongst themselves and with age. Thus some of the characters like cane height, tillering, length of leaf, girth of the stem, dry weight, and juice characters respond late in the life-cycle than early; quite reverse is the case with characters like photosynthesis, chlorophyll content, and respiration which show pronounced early response to fertilisers, as early as 45 days after germination. This is but in the expected sequence of events: that the response may be had in the morphological characters, dry matter production, and juice characters, it is essential that such predisposing internal factors as photosynthesis and respiration have an earlier response to fertilization. Growth after all is an integrated summation of photosynthesis and respiration; any manurial effect on increased growth, development and yield must necessarily be preceded by an early augmentative effect on both these contributory factors. A more intimate knowledge of the relation that added nutrients and related factors have with photosynthesis and respiration and through them with growth as a whole has thus to be looked for in any scientific scheme of crop production. Likewise in analysing the nature of fertiliser effect it is of increasing importance to have a clearer conception as to the nature and extent of the age factor in conditioning the intake of nutrients and the growth and development of the cane as a whole; also the relation it has with the various other internal and external factors with which the species is associated. A fuller treatment of these aspects would have to be reserved for later publication in this series.

Age effect.—With increase in age of the cane, there is the general tendency of increase in height which reaches a maximum when the canes are 250 days old (Fig. 1). The number of tillers on the other hand reaches the

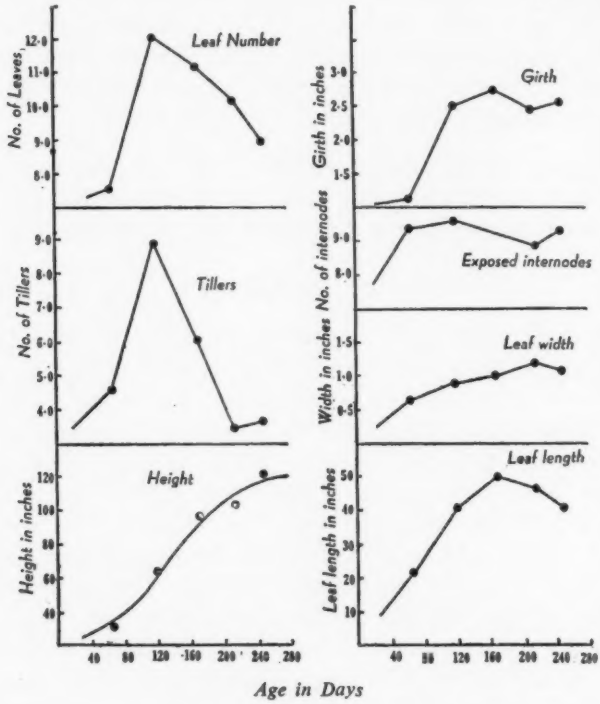

FIG. 1

Growth characters of Sugarcane (Co. 312) at Suceessive Stages of life-cycle

highest limit when the canes are about 120 days old; further advance in age brings about a decline. Similar is the case with the number of leaves on the main stem which attains the highest value 120 days after germination. The size of the leaf (length and breadth) also increase with advance in age and reaches a climax at an age of 170 and 215 days respectively. Number of exposed nodes increases till 120 days and remains more or less constant till the period of harvesting. So is the case with the girth of the stripped cane. Dry matter accumulation per plant also rises in a sigmoid fashion, reaching a maximum when the canes are 265 days old.

Judging from the sigmoid nature of the time-weight growth curve, and the rates of photosynthesis and respiration, the curves (Fig. 2) are clearly

distinguishable into two distinct phases.* In the case of the growth curve there is an inflection about the middle region of the curve when the plants are 135 days old. The first phase coincides with an age of 45–135 days in

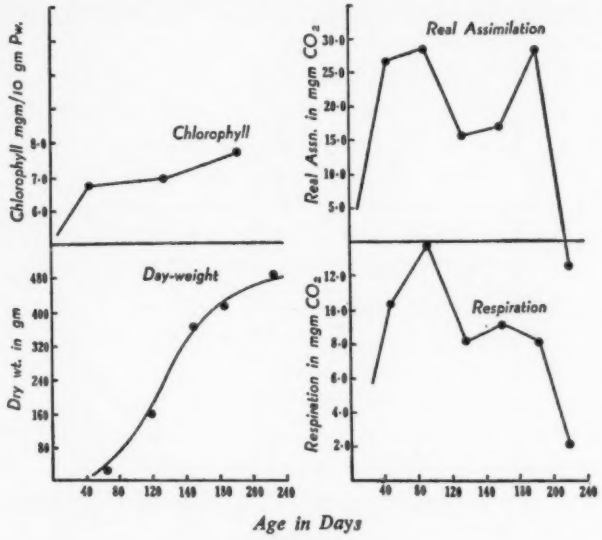

FIG. 2

Respiration, Assimilation, Chlorophyll and Dry Weight at Successive Stages of Growth

sugarcane. During this phase of the life-cycle, the general tendency of the crop is to show a gradually increasing efficiency of leaf in so far as assimilation and respiration are concerned, reaching a maximum and subsequently showing a decline. The second phase for growth, and respiration coincides with 135–260 days in the life-cycle. This period is also marked by more or less similar response of increase and decrease as noted for the earlier period of the life-cycle. On account of the characteristic nature of these curves the entire period of the life-cycle of the cane is divisible into the first half, *viz.*, 45–135 days, and second half, *viz.*, 135–260 days, and only the general response of the treatments discussed for the time with respect to these two periods of the life-cycle. The differences in the period of the attainment of the maximum efficiency of assimilation over the control, or its final cessation may largely depend upon the fertilisers added to the soil. The chlorophyll content of the leaf on the other hand does not show such

* For a fuller treatment of the subject reference may be made to an earlier publication: Singh, B. N., "The Basis of Ageing, Rejuvenation and Death," *Malaviya Commemoration Volume*, Indian Press, Allahabad, 1930.

phasic rise and fall but maintains a higher value at later stages of growth for all the fertilisers.

The data on the chemical composition of the juice indicate that the sucrose, °Brix and purity of the juice (Fig. 3) continue to increase with age

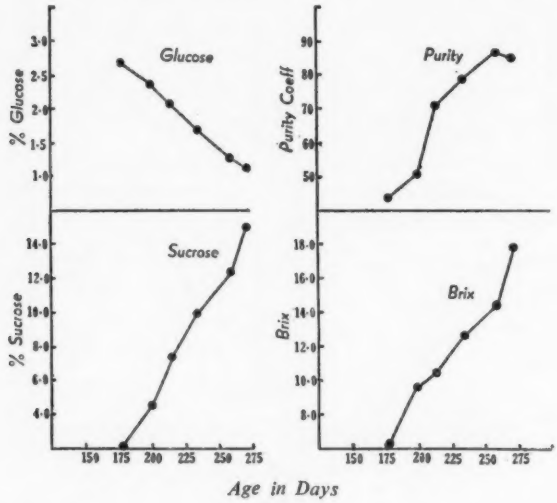

FIG. 3

Juice Characters of Sugarcane at Successive Stages of life-cycle

for all treatments; this is true for both the years of experimentation. Maximum sucrose is recorded for plants 274 days old and maximum purity at an age of 260 days. The percentage of glucose, however, declines with age reaching a very low value 274 days after germination. It may be noted that the period of highest sucrose coincides more or less with a stage when glucose is least and purity has attained a high level; this in fact may be the criterion for determining the stage earlier to which harvesting should not take place.

Fertiliser effects.—As has already been stressed in the preceding pages and also shown by other workers[2, 7, 41, 60, 63, 58] the problem of fertiliser effect is indeed a complex one. Of the three ingredients under study, nitrogen has been shown to be of greater value to the soils of this locality, but the problem of working out a nutritional balance sheet with respect to this ingredient is greatly handicapped by the difficulties arising out of a large number of variables at work in soil which influence the availability of nitrogen to the plant. Of these, the changes during nitrification and

immobilisation by micro-organisms, leaching, chemical reductions, removal by other agencies are but a few. Indeed evidences on this aspect of the problem have been adduced by many investigators[1, 11–14, 22 23, 26, 27, 35, 36, 38, 39] who have shown how largely the utility of the fertiliser depends upon such factors as the time of application, the form in which they are supplied, the specific ratio and combination with other component nutritional factors, and the species of crop plant under investigation.

The experimental evidences set forth in these pages, however, give indication to the view that there are certain specific ways in which a particular fertilising ingredient influences the growth characters of sugarcane plants. The responses noted in case of the specific doses that were used in these investigations (*cf.* p. 203) are summarised below:—

Nitrogen .. Increases height, number of leaves and girth of the stem; augments dry matter accumulation; increases assimilatory efficiency of leaves at all stages of life-cycle and respiration only during early stages; induces high yield of sugarcane but reduces juice purity.

Phosphorus .. Reduces the number of leaves and girth of the main stem; reduces assimilatory efficiency of leaves throughout the life-cycle; augments respiration during second half and diminishes it in the first half of the age-cycle.

Potash .. Increases assimilatory efficiency and respiration during first half, and decreases assimilation during the second half of the life-cycle. Purity is increased.

While these elements have been noted to exhibit significant responses with respect to the characters noted against each, the responses for other characters are either not significant or dissimilar during the two years of observation. Such characters have been purposely left out of consideration for the time. In this paper the facts as observed are stated as such: the significance of the results in the economics of fertiliser application and the relation that a single component growth factor may have with another will be discussed in the light of other work in contributions belonging to this series, which would include work on field experiments as well.

V. Summary

The investigation deals with the effects of sulphate of ammonia (N), superphosphate (P), and sulphate of potash (K) applied singly or in

combination at two levels each upon (i) height, tillering, leaf number, length and breadth of leaf, girth of stem, and nodes exposed; (ii) dry matter accumulation, photosynthetic and respiration rates, chlorophyll content and yield of stripped canes; and (iii) juice characters such as sucrose, glucose, °Brix, purity, and extraction percentage of sugarcane *var.* Co. 312.

The canes were grown in standard size internally waxed pots in farm soil (sandy loam) and the records with respect to the characters mentioned above taken at successive stages of the life-cycle of the crop. The data are subjected to statistical analyses. The following conclusions are the outcome of these researches:—

(i) The nature of fertiliser effect varies both with respect to the age of the plant and the fertilisers used. In some cases such as assimilation, respiration and chlorophyll the treatment effects are well marked at a very early stage of the life-cycle; in others such as height, tillering, length of the leaf, girth of the stem, dry matter accumulation, juice character, greater differences from treatment to treatment are noted towards the later stages of growth.

(ii) Increases in age, in general increases the magnitude of the various characters such as cane height, number of leaves, leaf size (length and breadth of leaves), exposed nodes, girth, and dry matter upto a certain stage of the life-cycle and later brings about a fall in the values. Photosynthesis and respiration rates on the other hand show two cyclic changes more or less similar in nature though differing in intensity, one during the early stages of growth (45–135 days) and the other during the later stages (135–240 days). The chlorophyll content of leaves on the contrary maintains a higher value at later stages. The differences in the period of attainment of maximum efficiency or its period of lull, however, depends largely upon the type of the fertiliser added to the soil.

(iii) The available evidences give indication to the view that there are certain specific directions in which the effect of a fertiliser is mostly felt. Thus with respect to the soil used and the specific doses of fertilisers applied, the responses to nitrogen, phosphorus, and potash are noted as under:—

Nitrogen .. Increases height, number of leaves and girth of the stem; augments dry matter accumulation; increases assimilatory efficiency of leaves at all stages of the life-cycle and respiration only during early stages; induces high yield of sugarcane but reduces juice purity.

Phosphorus .. Reduces the number of leaves and girth of the main stem; reduces assimilatory efficiency of leaves throughout the life-cycle; augments respiration during second half and diminishes it in the first half of the age-cycle.

Potash .. Increases assimilatory efficiency and respiration during first half and decreases assimilation during the second half of the life-cycle. Purity is increased.

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THE SHELL AND THE MECHANISM OF ITS CLOSURE IN THE INDIAN POND TERRAPIN, *LISSEMYS PUNCTATA PUNCTATA* (BONNATERRE)

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1. Introduction

THE recent species of the Trionychoid terrapins fall into two natural groups (Smith, 1931; Deraniyagala, 1939): (i) The *Cyclanorbidæ*, which are characterized by the presence of a femoral valve, under which the hind-limb may be concealed, the infra-costal position of the outer extremity of the nuchal bone, the fusion of the hyoplastron with the hypoplastron, and the occurrence of an ascending process on the posterior border of the pterygoid, and (ii) the *Trionychidæ*, which are characterised by the absence of the femoral valves, the supracostal position of the outer extremity of the nuchal bone, the distinctness of the hyoplastron from the hypoplastron and the absence of the ascending process on the pterygoid. Of these two groups, the first comprises only three genera, of which only one, *i.e.*, *Lissemys*, is found in India, Burma and Ceylon, while the other two (*Cyclanorbis* and *Cycloderma*)

are found in Tropical Africa. All these forms possess a shell more highly specialised for closure than any other testudinate, while the femoral valves constitute a unique feature in the whole order.

Although the shell of *Lissemys* and its development have been described by Boulenger (1890) and Deraniyagala (1939), nobody has so far studied in detail the anatomy and mechanism of the femoral valves, so characteristic of the *Cyclanorbidæ*. In the present paper the author deals with the shell of the Indian pond-terrapin *Lissemys punctata punctata*, and also describes the mechanism for the occlusion of the anterior openings for the head and the fore-limbs, the posterior openings for the hind-limbs, and the caudal opening for the tail.

2. *Material and Technique*

About two dozen specimens of *Lissemys punctata punctata* were employed for the present study, all obtained from the suburbs of Agra and properly identified for me by Mr. Beni Charan Mahendra. They varied in carapace length from 49 mm. to 255 mm.[1]

In the largest individuals the corselet was studied by dissection, while in the smaller ones the bones were studied by staining in alizarin and clearing in glycerin. The femoral valve and its hinge-joint, the marginals and their relation to the other parts, and the connection between the so-called 'callosities' and the plastral plates were examined in sections, prepared after decalcification in nitrated alcohol or even without it.

The muscles and ligaments of the movable parts of the shell were observed both in individuals dissected from the dorsal side as well as in those dissected from the ventral. The supra- and infra-caudal valves were studied in dissections and in sagittal sections. The functioning of the muscles and ligaments was interpreted by exerting requisite pulls on them after partial dissection in freshly chloroformed individuals.

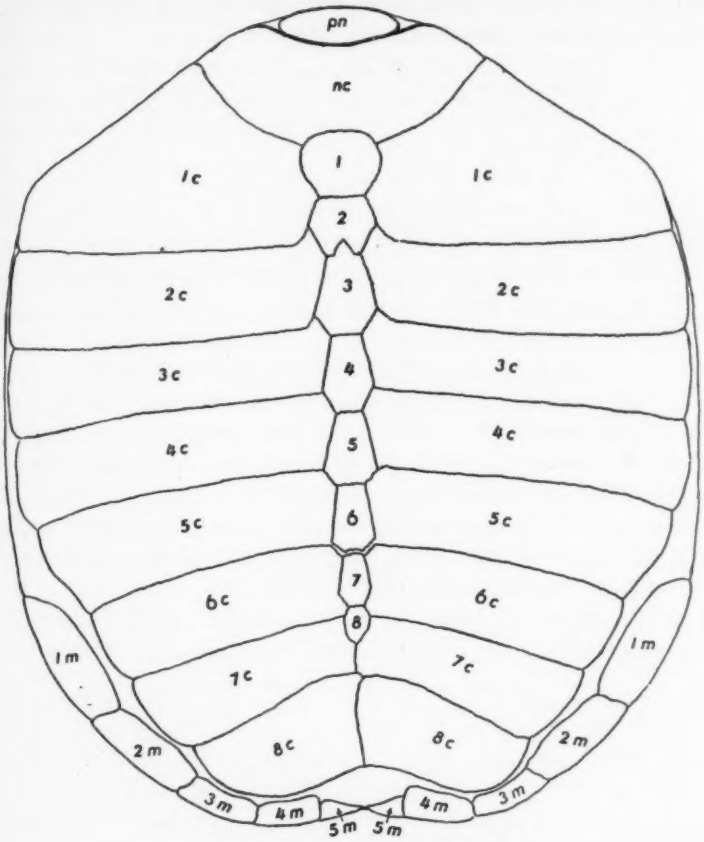
3. *The Corselet*

(*a*) *The Carapace:*

The carapace in *Lissemys punctata punctata* (Text-fig. 1) consists of a pre-nuchal,[2] a nuchal, eight neurals, eight pairs of costals, and a variable number of marginal plates. The *pre-nuchal* is a small, rounded and transversely elongated plate lying at the antero-mesial border of the carapace.

[1] The maximum length of carapace mentioned by Smith (1931) is 230 mm., the breadth being 170 mm. The largest specimen studied by me measured 255 mm. in carapace length, the breadth being 234 mm., *i.e.*, 25 mm. longer and 64 mm. broader than the maximum.

[2] Regarded as marginal by Smith (1931).



TEXT-FIG. 1. The Carapace (×$\frac{3}{4}$).

1–8 *c*, first to eighth costals; 1–5 *m*, marginals; 1–8, neurals; *nc*., nuchal; *pn*., prenuchal.

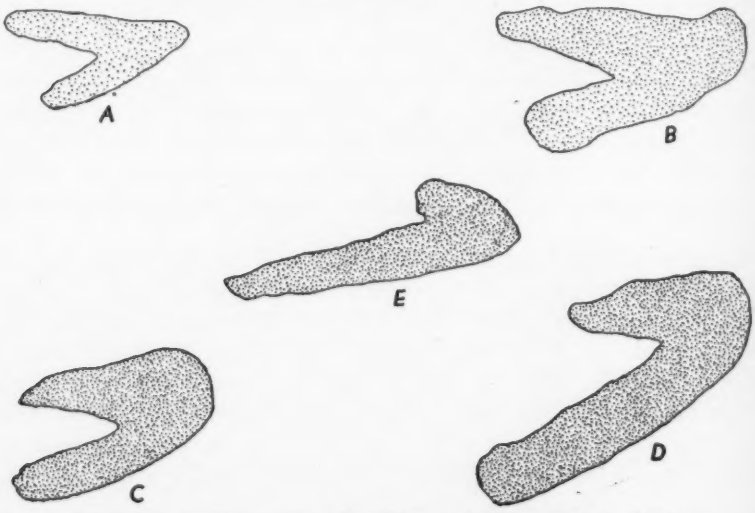
The *nuchal* is a fairly large bone, situated behind the pre-nuchal and characterized by a concave anterior border articulating with the pre-nuchal, the two being movably articulated till late in life. The *neurals*[3] and the *costals*, which are fused with the vertebrae and the ribs, are firmly connected with one another by sutures, and thus form a rigid structure within which internal movement is not possible; their number and arrangement are shown in Text-fig. 1. The *marginals* form a series on either side of the posterior part of the carapace opposite the sixth, seventh, and the eighth costals, and are connected

[3] Boulenger (1890) and Smith (1931) record 7 or 8 neurals for this genus; Deraniyagala (1939 finds them to be 8 to 6. In all specimens observed by me, the number was 8.

to them by thick dermis. Their number varies according to the size of the individual, as shown in the following table:

Carapace length	Number of marginals on each side
80 mm.	8
134 mm.	7
160 mm.	5
215 mm.	5
255 mm.	5

In this connection, it might be mentioned that Smith (1931) figures eight marginals on each side in *L. punctata scutata* (size of individual not given) and that Deraniyagala (1939) notes, in *L. punctata granosa*, " marginals twelve on each side subject to fusion which reduces their number to as low as eight ". In *L. punctata punctata*, however, the reduction, as shown in the foregoing table, goes down to five. The first marginal is the largest of the series both in *Lissemys punctata punctata* and in *L. punctata granosa*, while it is smaller than the second in *L. punctata scutata* (Smith, 1931). There is relatively a wide space betweeen the last pair of costals on one hand and the posterior marginals on the other. The last pair of marginals is extremely short and appears roughly triangular in shape in a dorsal view. It is connected to the adjacent marginals rather loosely. In transverse sections (Text-fig. 2) the marginals are <-shaped, with the angle between the two

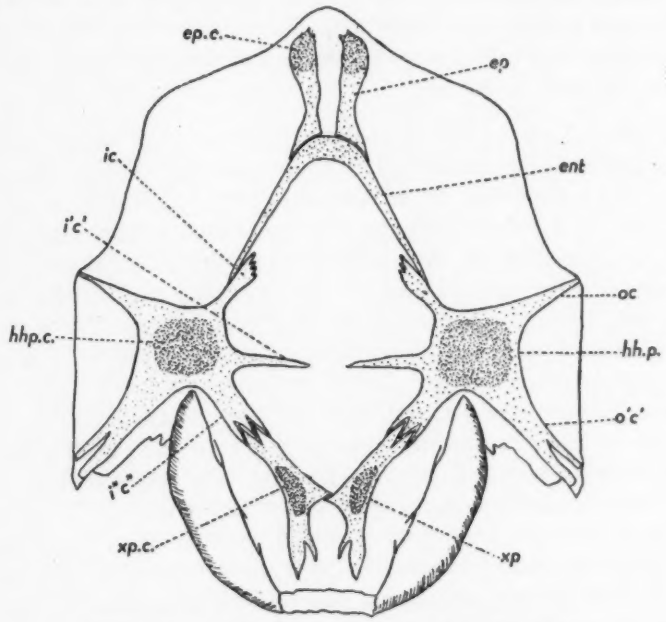


TEXT-FIG. 2. Transverse sections through various marginals (×2½).
A, first marginal; *B*, second marginal; *C*, third marginal; *D*, fourth marginal; *E*, fifth marginal.

arms of the < directed outwards, the space between the two arms faces the costals and the relative development of the two arms varies in different marginals. While both are well, though not quite equally, developed in the anterior ones, the upper arm is much reduced in the penultimate marginal and practically absent in the last one.

(*b*) *The Plastron :*

The plastron (Text-fig. 3) is composed of seven plates, *i.e.*, two *epiplastra*, one *entoplastron*, two *hyo-hypoplastra*, and two *xiphiplastra*. Each *epiplastron* is a small, dorso-ventrally flattened bone, slightly broadened behind



TEXT-FIG. 3. The plastron of a young individual (×1½).

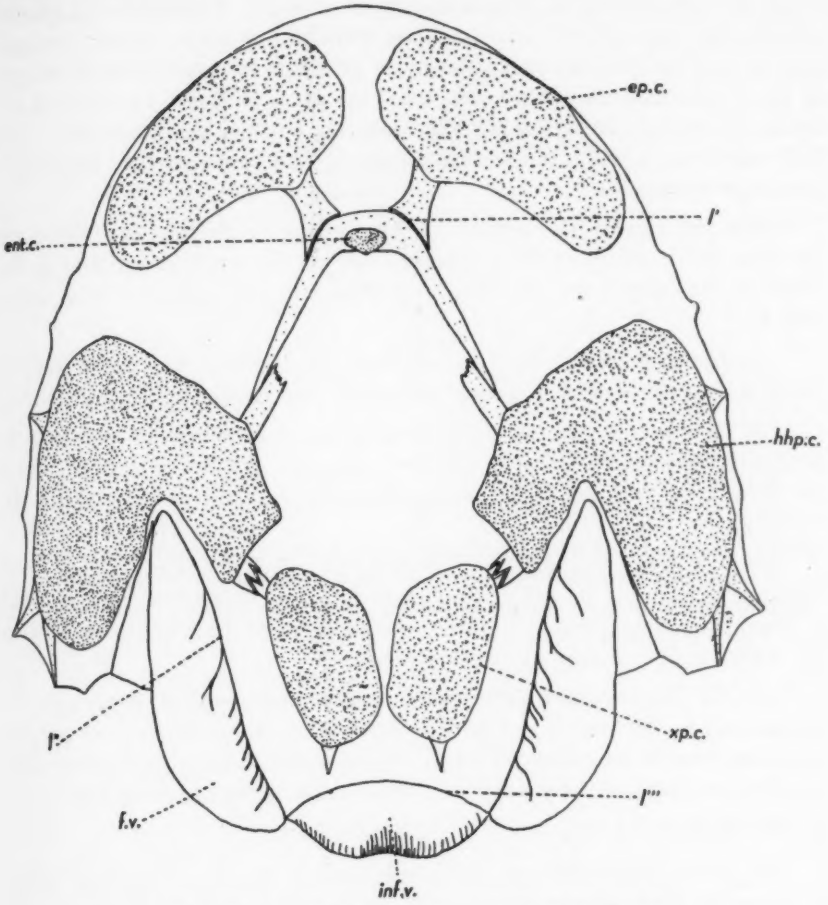
ent., entoplastron; *ep.*, epiplastron; *ep.c.*, epiplastral "callosity"; *hh.p.*, hyo-hypoplastron; *hhp.c.*, hyo-hypoplastral "callosity"; *ic*, *i'c'*, *i"c"*, inner cornua of the hyo-hypoplastron; *oc*, *o'c'*, outer cornua of hyo-hypoplastron; *xp*, xiphiplastron; *xp.c.*, xiphiplastral "callosity".

and applied to one of the arms of the entoplastron. It differs from the epiplastron of the *Trionychidæ* in being straight (not angular) and in the absence of a posterior limb. The *entoplastron* is shaped like an inverted V, with its angle rounded off; it articulates anteriorly with the posterior ends of the epiplastra and posteriorly with the outer border of the anteriormost inner cornu

of the hyo-hypoplastra. Each *hyo-hypoplastron* is a flat plate produced into five cornua: two from its outer border and three from its inner. Of the two outer cornua, the posterior is bifid distally, while the anterior is undivided. Of the three inner cornua, the anterior is connected with the arm of the entoplastron, and is frayed distally, the middle inner cornu is undivided, while the posterior is trifid distally. Each *xiphiplastron* is formed of three arms diverging from a central point. The anterior arm extends forwards and outwards, and bifurcates distally into two tooth-like processes, interlocking with the three processes of the posterior inner cornu of the hyo-hypoplastral plate. The posterior arm is directed sraight backwards, is bifid distally and ends freely in the dermis of this region. The middle arm, which projects from the inner border, articulates with the corresponding arm of the other xiphiplastral plate in such a way that the arm of the right plate comes to lie posteriorly to that of the left.

The plastral plates in Testudinates have been generally homologised with the clavicles, interclavicles and abdominal ribs of the other reptiles (Gadow, 1901; Reynolds, 1913; Kingsley, 1925; Williston, 1925). Their original form in *Lissemys punctata punctata* is clearly visible in very young alizarin-stained individuals, even as small as 49 mm. in carapace length. As growth proceeds, certain ossifications are developed superficially to, but in close osseous connection with, the original plastral plates. These are the so-called 'callosities' of previous workers. The term 'callosity', it must be pointed out, is inappropriate. These structures developed superficially to the homologues of clavicles, interclavicles and abdominal ribs, seem to correspond, in their development and location, to the dorsal pitted plates overlying the vertebrae and the ribs. In their early stages they are covered with skin, but become exposed when the cutaneous tissue over them is scraped off during the animal's movement on rough surface. It is proposed, therefore, to designate these structures as *extra-plastral plates* in order to distinguish them from the *original plastral plates*, which arise in a relatively deeper layer early in ontogeny. The extra-plastral plates, in all likelihood, correspond to the *epithecal layer* of Versluys (1914), while the plastral plates probably represent the *thecal layer*.

The *extra-plastral plates* in *Lissemys punctata punctata* (Text-fig. 4) are seven in number: two *extra-epiplastra*, one *extra-entoplastron*, two *extra-hyo-hypoplastra*, and two *extra-xiphiplastra*.



TEXT-FIG. 4. The plastron of an older individual (×1).

ent. c., entoplastral "callosity"; *f.v.*, femoral valve; *inf.v.*, infra-caudal valve; *l'*, hinge-line of the plastral lobe; *l''*, hinge-line of the femoral valve; *l'''*, hinge-line of the infra-caudal valve. (Other abbreviations as in Text-fig. 5.)

4. *Mobile Parts in the Corselet*

Deraniyagala (1939) describes the mode of occlusion of the corselet openings in *Lissemys punctata granosa* as follows :—

"The anterior plastral lobe is mobile, the posterior is fixed but possesses a fringing cutaneous valve which is movable. The line of flexure of the anterior lobe is immediately anterior to the entoplastral callosity and in young

specimens the prenuchal lobe is also freely mobile. Posteriorly the plastral valve, comprising two crural and one infra-caudal valve, presses upwards against the carapace in which the pygal area moves downwards to meet it, if the marginals are not too ossified. In spite of these structures in the adult every part cannot be retracted into the corselet simultaneously. The tail is depressed with a bluntly acuminate tip and barely extends beyond the carapace margin."

This description also applies to *Lissemys punctata punctata*. There are six lines of movement in this subspecies: two on the carapace and four on the plastron, resulting in the following six mobile parts in the corselet (Text-figs. 1 and 4) :

(*a*) *The Prenuchal Lobe* is movable on the nuchal bone, the movement being most pronounced in young specimens and obsolescent in older ones.

(*b*) *The Anterior Plastral Lobe*, forming the anterior part of the plastron, is adapted for the occlusion of the three corselet openings for the head and the fore-limbs, the line of movement lying between the epiplastra and the entoplastron.

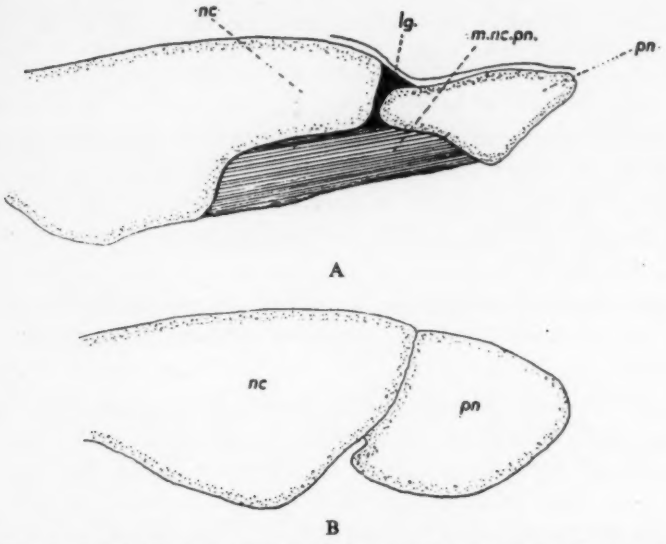
(*c*) *The Right and the Left Femoral* (*Crural*) *Valves* close up over the retracted hind-limbs, and their respective lines of movement are indicated by deep, obliquely-directed grooves, extending from the hyo-hypoplastra to the lateral edges of the infra-caudal valve.

(*d*) *The Supra-caudal Lobe*, forming the postero-mesial border of the carapace (the *pygal area*), can be flapped down to some extent over the tail and thus forms a supra-caudal valve, while the *Infra-caudal valve*, formed by the posterior edge of the plastron, serves to conceal the tail from below.

(*a*) *The Prenuchal Lobe :*

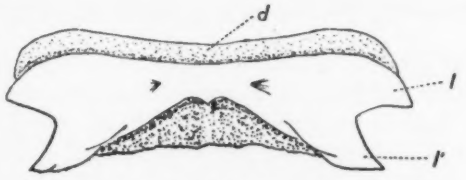
The *prenuchal lobe* (Text-fig. 5) is supported by the prenuchal bone, which is connected to the anterior border of the nuchal bone by a ligament until very late in life when this is replaced by suture. In all individuals up to 215 mm. in carapace length the prenucho-nuchal connection was still ligamental, while the largest individual examined by me (255 mm. in carapace length) showed a suture between these bones.

The nuchal (Text-fig. 6) shows two remarkable peculiarities in its ventral aspect, which have not been previously recorded and are due to the origin of muscles. In the first place, unlike the nuchal in other Indian Trionychoid terrapins (*Trionyx*, *Chitra*, etc.), it possesses a transversely directed groove along the ventral surface of its anterior border, serving for the origin of a short muscle inserted on the postero-ventral border of the prenuchal, the *Musculus*



TEXT-FIG. 5. Longitudinal section through the prenuchal and nuchal of (A) a medium-sized individual, and (B) of the individual 255 mm. in carapace length.

lg., ligament; *m.nc.pn.*, M. nucho-prenuchalis. (Other abbreviations as in previous figures.)



TEXT-FIG. 6. The nuchal (Ventral View) (×1).

d, depressed area; *l*, *l'*, lateral plates.

nucho-prenuchalis. The function of this muscle, not hitherto described, is to depress the prenuchal lobe over the retracted head. The groove, which also appears to have escaped attention, altogether disappears in very old individuals —an alteration apparently correlated with the loss of mobility of the prenuchal lobe and the atrophy of the nucho-prenuchal muscle.

Secondly, each outer ventral border of the nuchal juts out in the form of two thin lateral plates, which are separated from the thick dorsal ossification of this bone by a distinct groove, thus giving the bone a " butterfly-shaped " appearance. The posterior lateral plate extends obliquely backwards beneath the first costal in front of the second rib, while the anterior is directed

more or less outwards. These plates serve for the origin of the *nucho-epiplastral muscle*, described later.

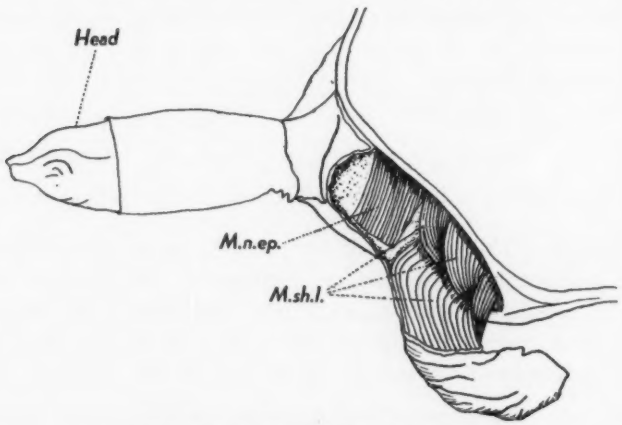
(*b*) *The Anterior Plastral Lobe :*

As already mentioned, the *anterior plastral lobe* serves to close the anterior openings of the shell when the head and the anterior limbs are withdrawn. It is roughly triangular in shape with its apex directed forwards, and its hinge-line lies across the anterior part of the plastron between the epiplastra and the entoplastron.

Careful observations leave no doubt that the mechanism for closing the plastral lobe is twofold: firstly by cutaneous tension, and secondly, by muscular action.

The skin covering the neck and the fore-limbs is continued behind on to the margin of the shell where it is attached dorsally to the anterior border of the carapace and ventrally to that of the plastron. When the head and the fore-limbs are retracted deep into the cavities accommodating them, a pull is naturally exerted by this skin on the border of the plastral lobe which tends to be raised up. This cutaneous tension, however, apparently, serves as a subsidiary aid in the occlusion, the main mechanism being muscular.

On each side of the base of the neck, there is a powerful muscle (Text-fig. 7), which arises from the lateral wing-shaped projections of the nuchal,



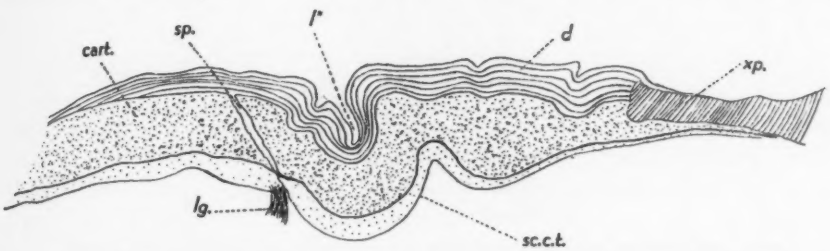
TEXT-FIG. 7. Lateral view of the neck.
M.sh.l., muscles of the shoulder-girdle and fore-limb; *M.n.ep.*, *M. nucho-epiplastralis.*

runs in a slightly oblique, dorso-ventral direction and is inserted on the epiplastral plate. We may designate it as *Musculus nucho-epiplastralis*. This

muscle is responsible for the firm closure of the plastral lobe. It appears to be absent in the Testudinates examined by previous workers (Bojanus,1819–21; Cuvier, 1835; Rathke, 1848; Owen, 1866; Hoffmann, 1890; Burne, 1905; Nishi, 1937, etc.).

(*c*) *The Femoral Valves*:

Each *femoral valve* (Text-fig. 4) is attached to the plastron along an obliquely directed line behind the posterior concave border of the hyo-hypoplastral plate and serves to conceal the hind-limb of its side when the latter is retracted. In transverse sections (Text-fig. 8) it is found to be composed of a stratified dermal layer overlying a supporting cartilage. The presence of



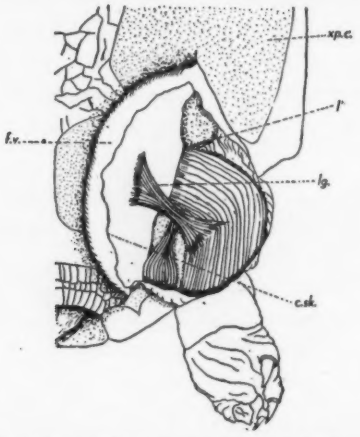
TEXT-FIG. 8. Transverse section through the femoral valve and the xiphiplastral region. *cart.*, supporting cartilage; *d*, dermis; *sc.c.t.*, subcartilaginous connective-tissue layer; *sp.*, split in cartilage. (Other abbreviations as in previous figures.)

such a cartilage in the femoral valves has not been previously recorded. The dermal tissue is several layers thick and is composed of longitudinally directed connective-tissue fibres. It is continuous with the dermal layer of the xiphiplastral region without a break even over the groove marking the articulation of the femoral valve. However, there is a clear split in the cartilaginous tissue underlying the dermis, not exactly under the line of articulation but a little outer to it. The presence of this split—another new feature—makes the ready movement of the femoral valve possible.

Connected with the femoral valve, a little behind its free margins, is the skin covering the hind-limb, and the closing of the femoral valve is, to some extent, due to the pull exerted on this skin (and thereby on the border of the valve) automatically by the retraction of the leg. Such skin tension, however, cannot be regarded as the sole (or even the main) cause of the occlusion of the femoral valve. Firstly, it was observed in living individuals that the skin is strained only when the leg has been withdrawn to its fullest extent, while the femoral valves start closing with the retraction of the leg even before complete retraction has taken place. Secondly, in freshly killed specimens

with a part of the carapace removed, the forcing of the leg inside closed the valves although the cutaneous connection had been carefully cut off from it.

More important than the skin in this connection is a peculiar ligament, not previously recorded (Text-fig. 9). It lies immediately beneath the skin and is attached, on the one hand, to the middle of the femoral valve and, on



TEXT-FIG. 9. Dissection to show the ligament of the femoral valve (×1).

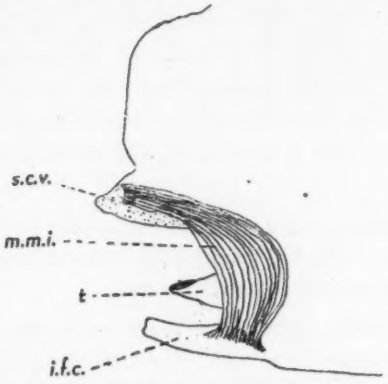
c.sk., line of attachment of skin to the valve; *lg.*, ligament connecting the femoral valve to the shank; *f.v.*, inner aspect of the femoral valve, visible as the valve is flapped over after cutting the skin attached to it. (Other abbreviations as in previous figures.)

the other, to the shank. As the shank is drawn in during the retraction of the leg, this ligament is pulled along with it, dragging the femoral valve and closing it tightly on the crural orifice of the corselet.

(*d*) *The Supra- and Infra-caudal Valves :*

The tail is extremely short and ends well within the posterior margin of the corselet. It is very feebly retractile and can be entirely concealed by the closing together of the supra- and the infra-caudal valves, of which the latter is definitely more mobile. The *supra-caudal valve* is really the projecting posterior border of the carapace, supported mainly by the last pair of marginals, although the penultimate pair of marginals also contributes to its formation. The *infra-caudal valve* is a flap-like structure developed on the plastron beneath the tail and bears a concave border posteriorly. The skin covering the outer surfaces of both these valves is reflected at the rim to become continuous with the skin of the tail, but it is rather loose between them and the caudal region—a provision apparently for the closure of the valves and the retractility (although slight) of the tail.

When the skin is carefully cut off and a part of the carapace and plastron removed so as to display the internal relations between the valves and the tail, a peculiar muscle (Text-fig. 10) is found embracing the lateral and ventral aspects of the tail on each side. This muscle arises on the last pair of



TEXT-FIG. 10. Lateral dissection showing the muscle of the caudal orifice (semi-diagrammatic).

i.f.c., infra-caudal valve; *m.m.i.*, margino-infra-caudal muscle; *s.c.v.*, supra-caudal valve; *t.*, tail.

marginals and after running obliquely outwards, is inserted on the dorso-lateral surfaces of the infra-caudal valve. Its contraction is responsible for the pressing together of the supra- and the infra-caudal valves and the consequent closure of the caudal orifice. I have not found it possible to homologise this muscle with any of the caudal muscles described by previous authors (Bojanus, 1819–21; Meckel, 1828; Owen, 1866; Hoffmann, 1890) and think it justifiable provisionally to designate it *M. margino-infracaudalis*, until a careful comparative study of other testudinate genera makes it possible to identify it properly.

5. *Summary*

The author describes in detail the mechanism for the occlusion of the corselet orifices in *Lissemys punctata punctata*. The following features described by him are new :

(1) The marginals, unlike those in *Lissemys punctata granosa*, are reduced to five. They are < -shaped, but differ amongst themselves in the relative development of the two arms.

(2) The so-called ' callosities ' on the plastron are not real callosities, but represent *extra-plastral plates*, belonging perhaps to the *epithecal layer* of Versluys.

(3) The lines of movement in the shell are described in detail and it is shown that there are six mobile parts.

(4) The prenucho-nuchal connection is ligamental in all individuals up to 215 mm. in carapace length, but becomes sutural later on.

(5) The nuchal shows a transverse groove at the anterior border of its ventral surface and two wing-like projections. The groove serves to accommodate a muscle, the *musculus nucho-prenuchalis*, not described before, while the lateral projections serve for the origin of a muscle inserted on the plastron.

(6) The prenuchal lobe is moved by the *nucho-prenuchalis muscle*, not hitherto described.

(7) The mechanism of the anterior plastral lobe has been studied. It is found that its closure is effected partly by cutaneous tension, but mainly by a new muscle, the *musculus nucho-epiplastralis*.

(8) The femoral valves possess a cartilage inside and are connected to the shank by a ligament, not so far known. The closing of these valves is effected by the drawing in of this ligament during the retraction of the leg.

(9) The tail is extremely short and is very feebly retractile.

(10) A peculiar muscle—*M. margino-infracaudalis*—arises from the last pair of marginals and is inserted on the infra-caudal valve. This is responsible for the closure of the caudal orifice.

6. *Acknowledgments*

The problem was suggested to me by Mr. Beni Charan Mahendra under whose guidance the present work has been carried out. I am very grateful to him for his painstaking help and constructive criticism. My thanks are also due to the authorities of St. John's College for the facilities I have enjoyed in the Department.

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ON A COLLECTION OF ALGAE FROM THE CHAMBA STATE, PUNJAB—I

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[Communicated by Professor Y. Bhâradwâja, M.Sc. Ph.D. (London), F.L.S., F.N.I.]

While on a botanical excursion to the Punjab hills during the last Puja Vacation in company with Professor Bhâradwăja, the writer had the opportunity to collect and study the algal flora of Chamba, the capital of the Chamba State, and a part of the Ravi valley. Practically nothing is known about these lower plants of this beautiful and almost unexplored part of the country. Even the phanerogamic vegetation is not completely known, although there is certain scattered information given by Dr. T. Thomson in 1848 and Sir G. Watt in 1878, as recorded in the " Punjab States' Gazetteer," Volume XXII A, Chamba State, 1904. It has been planned to describe the algal flora of this part of the country in a series of papers. The present communication deals with some of the Myxophyceae and the Chlorophyceae. In all twenty-one forms have been described and out of these twelve are blue-greens and nine belong to the Chlorophyceae. Five forms are new.

The capital of Chamba is situated at a height of 3,027 feet above sea level, on the bank of the river Ravi, and the climate is therefore distinctly sub-tropical. Trees and plants of European type are scarcely seen, while Indian forms predominate, such as the *Euphorbias*, *Bauhinias*, *Cassias* several thorny *Acacias*, species of *Ficus* and bamboos. The algal flora of the country at the time of collection was very scant and mostly confined to the Ravi. The river itself hardly harbours any algae excepting a few *Cladophoras* (*C. glomerata* and *C. crispata*) fast attached to the bed along with sterile species of *Spirogyra*, which later on become free-floating. The free-floating forms are exclusively absent. But, frequently the river is dammed by the people and outlets are maintained for domestic purposes (for running flour-mills, etc.). As the river recedes after the rains, the stagnant waters of low-lying areas often contain a thick and shining green covering of *Tetrasporidium javanicum* on the surface.

Systematic Enumeration of the Species Observed

MYXOPHYCEAE

Chroococcales

Chroococcaceae

Genus *Merismopedia* Meyen.

1. *Merismopedia punctata* Meyen. Geitler in Rabenhorst's *Kryptogamen-Flora von Europa*, Band XIV, *Cyanophyceae* 1930–32, p. 264, Fig. 129 *c*.

Lat. cell., 2·5–3·1 μ.

Habitat.—Along with sterile filaments of *Spirogyra* sp. in stagnant water in the receding bed of the river Ravi. (Oct., 1940)

Hormogoneales

Rivulariaceae

Genus *Calothrix* Agardh

2. *Calothrix fusca* Born. et Flah. Frémy in Les Myxophycées de l' Afrique équatoriale francaise, *Arch. d. Bot.*, iii (1929), Mem. 2, 1930, p. 246, Fig. 222; Geitler, *op. cit.*, 1930–32, p. 611, Fig. 384.

Lat. fil. at the base., 10–16·2 μ; lat. fil. in the middle, 10–12·1 μ; lat. trich. in the middle, 7·2–8·3 μ.

Habitat.—In the mucilage of *Nostoc verrucosum* Vauch., in the receding bed of the river Ravi. (Oct., 1940)

Scytonemataceae

Genus *Scytonema* Agardh

3. *Scytonema Iyengari* Bhâradwâja. Bhâradwâja, " The Taxonomy of *Scytonema* and *Tolypothrix* including some new records and new species from India and Ceylon," *Revue Algologique*, 1933, n. 1–2, p. 162, Fig. 2, C–D.

Forma

Lat. fil., 19·2–23 μ; lat. trich., 11·5–15·4 μ; long. cell., 8–13·4 μ; crass. vag., 2·1–4 μ; lat. het., 11·5–13·5 μ; long. het., 17·2–51·8 μ.

Habitat.—On stone in the receding bed of the river Ravi. (Oct., 1940)

The form differs from the type in the pseudobranches being many and not necessarily short and in the sheath which does not take chloro-zinc iodide stain.

Nostocaceae

Genus *Cylindrospermum* Kuetz

4. *Cylindrospermum muscicola* Kuetz. var. *kashmirensis* Bhard. Bhâradwâja, "Contributions to our knowledge of the Myxophyceae of India," *Ann. Bot.*, 47, 185, 1933, pp. 123–31, Figs. 1–2.

Forma

Lat. cell., 3·8–4 μ; long. cell., 3·8–4·5 μ; lat. het., 4·6–5·7 μ; long. het., up to 7·7 μ; lat. spor., 5·7–7·7 μ; long. spor., 9·4–23 μ.

Habitat.—In stagnant road-side ditch, on Chil to Chamba road. (Oct. 1940)

The form differs from the type in the longer spores and shorter heterocysts.

Genus *Nostoc* Vauch.

5. *Nostoc verrucosum* Vauch. Geitler, *op. cit.*, 1930–32, p. 854, Fig. 542.

Lat. cell., 3–3·2 μ; lat. het., up to 3·8 μ; lat. spor., 3·8–5 μ; long. spor., 5·3–7·7 μ.

Habitat.—On moist rock, Chamba. (Oct., 1940)

Oscillatoriaceae

Genus *Oscillatoria* Vaucher

6. *Oscillatoria sub-brevis* Schmidle. Geitler, *op. cit.*, 1930–32, p. 946, Fig. 601 *b*.

Lat. cell., 4·9–6·8 μ; long. cell., 1·5–2·5 μ.

Habitat.—Along with *Cladophora fracta* on moist rock, on the bank of the river Ravi. (Oct., 1940)

7. *Oscillatoria terebriformis* Agardh. *Forma* Rao. Rao, "The Myxophyceae of the United Provinces, India—II," *Proc. Ind. Acad. Sci.*, 1936, 3, No. 2, Sec. B, p. 171, Figs. 1–2.

Lat. trich., 4–4·6 μ; long. cell., 1·5–2·5 μ.

Habitat.—On moist ground near the bank of Ravi. (Oct., 1940)

8. *Oscillatoria Agardhii* Gom. Geitler, *op. cit.*, 1930–32, p. 974, Fig. 621.

Lat. trich., 5·7–7·6 μ; long. cell., 2·5–5·4 μ.

Habitat.—On moist ground near the bank of the river Ravi. (Oct., 1940)

9. *Oscillatoria deflexa* West var. *crassa* Woronich. Geitler, *op. cit.*, 1930–32, p. 975.

Lat. cell., 1·9 μ.

Habitat.—On moist ground on the bank of the river Ravi. (Oct., 1940)

Genus *Lyngbya* Agardh

10. *Lyngbya polysiphoniae* Frémy. Frémy, *op. cit.*, 1930, p. 197, Fig. 161.

Forma

Lat. fil., up to 2·2 μ; long. cell., 0·73–1·1 μ.

Habitat.—On moist rock on the bank of the river Ravi. (Oct., 1940)

The form differs from the type in much longer filaments and its habit.

11. *Lyngbya Martensiana* Menegh. Frémy, *op. cit.*, 1930, p. 193, Fig. 158.

Forma

Lat. fil., 8·4–11·5 μ; lat. trich., 6·9–7·7 μ; long. cell., 1·2–1·5 μ.

Habitat.—Along with *Cladophora* spp., Chamba. (Oct., 1940)

The form differs from the type in the filaments being single and in the cells being shorter.

Genus *Microcoleus* Desmazieres

12. *Microcoleus chthonoplastes* Thur. Geitler, *op. cit.*, 1930–32, p. 1134, Fig. 739.

Lat. cell., 3·8–4·9 μ; long. cell., 3·8–5·7 μ.

Habitat.—On moist soil, Chamba. (Oct., 1940)

CHLOROPHYCEAE

VOLVOCALES

Tetrasporineae

Palmellaceae

Genus *Tetrasporidium* Mœbius

13. *Tetrasporidium javanicum* Mœbius. Iyengar, "Two little known genera of green algae (*Tetrasporidium* and *Ecballocystis*)," *Ann. Bot.*, 46, 1932, p. 191–227, Figs. 1–3.

Diam. cell., 10–11·5 μ.

Habitat.—Free-floating in glistening green masses in the receding bed of the river Ravi. (Oct., 1940)

CHLOROCOCCALES

Hydrodictyaceae

Genus *Hydrodictyon* Roth.

14. *Hydrodictyon reticulatum* (L.) Lagerheim. Lemmermann, Brünthaler und Pascher in Pascher's *Süsswasser-Flora Deutschlands Österreichs und Der Schweiz*, Heft 5, 1915, p. 166, Fig. 68.

Long. coenocyte., up to 307 μ.

Habitat.—In stagnant ditch, on Chil-Chamba road. (Oct., 1940)

ULOTRICHALES

Ulotrichaceae

Genus *Ulothrix* Keutz.

15. *Ulothrix tenerrima* Kuetz. Heering in Pascher's *Süsswasser-Flora Deutschlands Österreichs und Der Schweiz*, Heft 6, 1914, p. 33, Figs. 28–30.

Lat. cell., 8·4–9·6 μ; long. cell., 7·5–9·6 μ.

Habitat.—In a stagnant ditch, on Chil-Chamba road. (Oct., 1940)

16. *Ulothrix zonata* Kuetz. Heering, *op. cit.*, Heft 6, 1914, pp. 34–35, Figs. 35–36.

Lat. cell., 19·2–46 μ.

Habitat.—In a stagnant ditch, on Chil-Chamba road. (Oct., 1940)

CLADOPHORALES

Genus *Rhizoclonium* Kuetz.

17. *Rhizoclonium hieroglyphycum* (Agardh.) Kuetz. Heering, *op. cit.*, Heft 7, 1914, p. 22, Fig. 21 *a–c*.

Lat. cell., 10–38 μ; lat. vag., up to 3 μ.

Habitat.—Attached to submerged rocks along with *Cladophora glomerata* in a swift-flowing rivulet, Chamba. (Oct., 1940)

Genus *Cladophora* Kuetz.

18. *Cladophora glomerata* (L.) Kuetz. Heering, *op. cit.*, Heft 7, 1914, p. 37, Fig. 39.

Lat. fil. main., 103·7 μ.

Habitat.—Attached to submerged rocks in the bed of the river Ravi. (Oct., 1940)

19. *Cladophora glomerata* (L.) Kuetz. var. *simplicior* Brand. Fritsch, "The Structure and Reproduction of the Algae," 1935, Vol. I, p. 230, Fig. 68, K.

Lat. fil. main., 49–76·8 μ.

Habitat.—Attached to submerged rocks in the bed of the river Ravi. (Oct., 1940)

20. *Cladophora crispata* (Roth.) Kuetz. Heering, *op. cit*., Heft 7, 1914, p. 40, Fig. 40 *a*.

Lat. fil., main., up to 125 μ.

Habitat.—Attached to submerged rocks in the bed of the river Ravi. (Oct., 1940)

SIPHONALES

Vaucheriaceae

Genus *Vaucheria* De Candolle

21. *Vaucheria sessilis* De Candolle. Heering, *op. cit*., Heft 7, 1914, p. 88, Fig. 75.

Forma

Lat. fil., 38·4–92·2 μ; crass. oog., 61·4–72·9 μ; long. oog., 69·1–80·6 μ; crass. oosp., 65·3–88·3 μ; long. oosp., 61·4–76·8 μ.

Habitat.—On moist soil, Chamba. (Oct., 1940)

The form differs in the much broader filaments.

In conclusion, I have much pleasure in expressing my great indebtedness to Professor Bhâradwâja, for his kind guidance and criticism throughout the course of this investigation.

THE CHLOROPHYCEAE OF THE BENARES DISTRICT, INDIA—I

By Vijay Pratap Singh, M.Sc.

(From the Department of Botany, Benares Hindu University)

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[Communicated by Prof. Y. Bhâradwâja, M.Sc., Ph.D. (London), F.L.S., F.N.I.]

The present communication deals with some of the Chlorophyceæ collected from Benares and its environs during the years 1939, 1940 and 1941. In all twenty-one forms representing sixteen genera have been described, and out of these three forms are new.

Systematic Enumeration of the Species Observed

Chlorococcales

Hydrodictyaceae

Genus *Hydrodictyon* Roth.

1. *Hydrodictyon reticulatum* (L.) Lagerheim. Lemmermann, Brünnthaler und Pascher, in Pascher's *Süsswasser-Flora Deutschlands Österreichs und Der Schweiz*, Heft 5, 1915, p. 106, Fig. 68.

Lat. coenocyte., 168·9–192 μ; long. coenocyte., up to 3 mm.

Habitat.—In a stagnant shallow pond, Bawanpokhari. (Dec. 1940)

Ulotrichales

Ulotrichaceae

Genus *Ulothrix* Kuetzing

2. *Ulothrix zonata* Kuetz. Heering, in Pascher's *Süsswasser-Flora Deutschlands Österreichs und Der Schweiz*, Heft 6, 1914, pp. 34 and 35, Figs. 35–36.

Lat. cell., 18·4–43 μ.

Habitat.—In a shallow pond, Bawanpokhari. (Feb. 1941)

Ulvaceae

Genus *Enteromorpha* (Link) Harvey

3. *Enteromorpha prolifera* Agardh. Heering, *op. cit.*, Heft 6, 1914, p. 26, Fig. 24.

Forma

Diam. cell., 12·2–16·7 μ.

Habitat.—In a shallow pond, Bawanpokhari. (Feb., 1941)

The form differs from the type in the broader cells.

CLADOPHORALES

Cladophoraceae

Genus *Rhizoclonium* Kuetzing

4. *Rhizoclonium hieroglyphycum* (Agardh) Kuetz. Heering, *op. cit.*, Heft 7, 1914, p. 22, Figs. 21, *a*, *b*, *c*.

Lat. fil., 26·9–30·7 μ.

Habitat.—In a stagnant pond, Bawanpokhari. (Feb., 1941)

Genus *Cladophora* Kuetzing

5. *Cladophora crispata* (Roth.) Kuetz. Heering, *op. cit.*, Heft 7, 1914, p. 40, Fig. 40.

Lat. fil. main., 91·1 μ.

Habitat.—Along with *Cladophora fracta* Kuetz., Bawanpokhari. (Oct., 1940)

6. *Cladophora fracta* Keutz. Heering, *op. cit.*, Heft 7, 1914, p. 43, Fig. 41.

Lat. fil. main., 88·3–115·7 μ.

Habitat.—Along with *Cladophora crispata*, Bawanpokhari. (Oct., 1940)

CHAETOPHORALES

Chaetophoraceae

Genus *Chaetophora* Schrank

7. *Chaetophora elegans* (Roth.) Agardh. Heering, *op. cit.*, Heft 6, 1914, p. 94, Figs. 137–40.

Lat. cell., 6·4–9·6 μ; long. cell., up to 5·8 μ.

Habitat.—Epiphytic on dead aquatic leaves, Botanical Garden Pond. (Jan., 1939)

Genus *Stigeoclonium* Kuetzing

8. *Stigeoclonium tenue* Kuetz. (Sammelart). Heering, *op. cit.*, Heft 6, 1914, p. 79. Fig.

Lat. cell., 6–8·7 μ; long. cell., up to 22 μ.

Habitat.—Epiphytic on *Oedogonium* sp., Bawanpokhari. (Dec., 1939)

Trentepohlieae

Genus *Trentepohlia* Martius

9. *Trentepohlia odorata* (Wiggers) Wittrock. Heering, *op. cit.*, Heft 6, 1914, p. 120, Fig. 174.

Lat. cell., 7·7–15·3 μ; long. cell., 11·5–21·2 μ; lat. gametan., 8·4–19·2 μ; long. gametan., 19·2–30·7 μ.

Habitat.—On the bark of *Mangifera indica*, Bawanpokhari. (Feb., 1941)

Coleochaetaceae

Genus *Coleochaete* Brebisson

10. *Coleochaete pulvinata* Braun. Heering, *op. cit.*, Heft 6, 1914, p. 132, Figs. 187–88.

Lat. cell., 15·4–19·2 μ; long. cell., up to 28 μ; crass. oosp. cum vag., 72·9–96·2 μ; crass. oosp., 42·3–57·6 μ.

Habitat.—Epiphytic on *Hydrilla* leaves, Bawanpokhari. (Feb., 1941).

The alga differs from the type in the narrow vegetative cells and oospores.

11. *Coleochaete scutata* Brebisson. Heering, *op. cit.*, Heft 6, 1914, p. 136, Fig. 194.

Lat. cell., 11·5–26·9 μ; long. cell., up to 40 μ; crass. oosp., 50·1–57·6 μ; long. oosp., 61·4–71·2 μ.

Habitat.—Epiphytic on *Hydrilla* leaves, Bawanpokhari. (Feb., 1941)

The alga differs from the original type in shorter cells and oospores.

Pleurococcaceae

Genus *Pleurococcus* Meneghinii

12. *Pleurococcus Naegelii* Chodat. Fritsch, *The Structure and Reproduction of the Algae*, Vol. I, 1935, p. 288, Fig. 90, *d–h*.

Lat. cell., 8·4–12·2 μ; long. cell., 7·7–9·6 μ.

Habitat.—On the bark of a tree, Bawanpokhari. (Feb., 1941)

CONJUGALES

Zygnemoideae

Genus *Zygnema* Agardh

13. *Zygnema normani* Taft. var. *lamellata* Rao. Rao, "The Zygnemoideae of the United Provinces, India—I," *Jour. Ind. Bot. Soc.*, Vol. 16, No. 5, 1937, pp. 269–88, Figs. 2, A and B.

Forma

Lat. cell., 26·8–32·6 μ; long. cell., 38·4–96 μ; lat. zygosp., 30·7–38·4 μ; long. zygosp., 31·4–57·6 μ.

Habitat.—Free-floating in Bawanpokhari. (Feb., 1940)

The form differs from the type in much broader filament and in the many narrower scrobiculations on the mesospore.

Genus *Spirogyra* Link

14. *Spirogyra Weberi* (Kuetz.) Czurda. Czurda in Pascher's *Süsswasser-Flora Mitteleuropas*, Heft 9, 1932, p. 154, Fig. 153.

Forma

Lat. cell., 42·3–46·6 μ; long. cell., 212–39 μ; lat. zygosp., 42·2–48·6 μ; long. zygosp., 75–92 μ.

Habitat.—Free-floating in Bawanpokhari.

The form differs from the type in broader cells and much bigger zygospores.

Genus *Sirogonium* Kuetz.

15. *Sirogonium strictum* Kuetz. Czurda, *op. cit.*, Heft 9, 1932, p. 144, Fig. 142.

Lat. cell., 42·2–46 μ; long. cell., up to 4 times; lat. zygosp., 53·8–65·8 μ; long. zygosp., 76·8–107·5 μ.

Habitat.—Free-floating in Bawanpokhari.

SIPHONALES

Vaucheriaceae

Genus *Vaucheria* De Candolle

16. *Vaucheria uncinata* Kuetz. Heering, *op. cit.*, Heft 7, 1914, p. 91, Fig. 82.

Lat. fil., 70–96·8 μ; crass. oosp., 65–76 μ; long. oosp., 20–76·8 μ.

Habitat.—On the bank of river Barna.

CHARALES

Nitelleae

Genus *Nitella* Agardh

17. *Nitella hyalina* (De Candolle) Agardh. Groves and Bullock-Webster, *British Charophyta*, 1920, Vol. I, Pl. XVI; Pal, "Burmese Charophyta," *Jour. Linn. Soc. Bot.*, 1932, Vol. XLIV, No. 327, p. 67, Pl. 8;

Dixit, "Charophyta of Bombay Presidency—II," *Jour. Ind. Bot. Soc.*, Vol. XVIII, Nos. 4–6, 1940, pp. 231–39.

Diam. anth., 265–364·2 μ; crass. oog., 324–96 μ; long. oog., 257·9–396·4 μ; crass. oosp., 265–80 μ; long. oosp., 200–65 μ.

Habitat.—Bawanpokhari.

Chareae

Genus *Chara* Linnaeus

18. *Chara corallina* Willdenow. Pal, *op. cit.*, 1932, p. 80; Dixit, *op. cit.*, 1940, p. 235.

Lat. stem., up to 850 μ; lat. anth., 648–747·9 μ; long. anth., 664·8–914·1 μ; lat. oog., 998·7–1163·4 μ; long. oog., 1180–1498 μ; lat. oosp., 997·9–1160 μ; long. oosp., 1160–1495 μ.

Habitat.—Bawanpokhari.

19. *Chara Wallichii* Braun. Pal, *op. cit.*, 1932, p. 79, Pls. 14 and 15.

Lat. stem., up to 900 μ; lat. anth., 512·2–947·5 μ; long. anth., 531·8–997 μ; lat. oosp., 965·9–1030 μ; long. oosp., without cornula., 1030–1246 μ.

Habitat.—Bawanpokhari.

20. *Chara Brachypus* Braun. Pal, *op. cit.*, 1932, p. 87; Dixit, *op. cit.*, 1940, pp. 237–38.

Lat. stem., 450–500 μ; diam. anth., 320–50 μ; crass. oog., 540–600 μ; long. oog., 760–835 μ; lat. oosp., 350–400 μ; long. oosp., 389–550 μ.

Habitat.—Bawanpokhari.

21. *Chara zeylanica* Willdenow. Pal, *op. cit.*, 1932, p. 88; Dixit, *op. cit.*, 1940, p. 238.

Lat. stem., 650–795·5 μ; diam. anth., 280–320 μ; crass. oog., 400–540 μ; long. oog., 700–900 μ; crass. oosp., up to 380 μ; long. oosp., 580–700 μ.

Habitat.—Bawanpokhari. (Feb., 1941)

In conclusion, I have much pleasure in expressing my great indebtedness to Professor Bharadwaja, for his kind guidance and criticism throughout the course of this investigation.

THE ALTERATION OF RADIOACTIVE MINERALS

By T. C. Sarkar, M.Sc. (Cal.)

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The soundness of the "lead-ratio" method of determining the age of a radioactive mineral (and hence of the formation where it occurs) is well established, as the lead ratios obtained from different radioactive minerals from the same locality have been practically identical in many cases. None-theless it has been observed by different workers that different radioactive minerals from the same locality, and sometimes from the same pegmatite, indicate different ages; also that the uranium minerals generally indicate a higher age than the thorium minerals and these latter have lead ratios varying greatly among themselves. The case of thorium minerals from Norway and Ceylon is prominently illustrative.

From a study of lead accumulated in thorium minerals Boltwood[1] and later Holmes and Lawson[2] came to the conclusion that lead was not the end product of the disintegration of thorium, and even if it was so, a part only of the lead was stable and the remainder further disintegrated into thallium. Soddy[3] and Lawson[4] considered that the thorium minerals were of a secondary nature and necessarily gave lower age indications than the primary minerals with which they were associated. Against this, Joly[5] appeared to have found evidence that uranium might have disintegrated more rapidly in earlier times than to-day, and consequently uranium minerals would give higher age indications than normal, whereas thorium minerals disintegrating at a constant rate would give correct age values. None of these ideas have been found to be satisfactory and they have been dropped in course of time. The formulation of the above ideas shows the complex nature of the puzzle and how the disagreeing lead ratios of radioactive minerals have affected the development of the fundamentals of radioactivity.

In the opinion of the present writer, the essential condition about the unaltered nature of the mineral was lost sight of by many investigators. We shall consider in this paper how the radioactive minerals undergo alteration and how the lead ratio of such minerals are affected in consequence of the process of alteration. We shall also discuss some relevant points in the light of the ideas presented here.

The radioactive minerals have a great tendency to occur in the metamict state. This phenomenon was first observed by Brögger[6] in the case of the rare earth minerals and was thought by him to be due to hydration. Later Mügge[7] considered that the radioactive substances occurring in these minerals by their disintegration were responsible for the metamict state of the minerals. Goldschmidt[8] noted that minerals that usually occur in the metamict state are mostly combinations of weak acids and weak bases and according to him, the percolating waters cause hydrolysis in the minerals, the components of which then exist as a solid solution of the oxides, and consequently the minerals occur in the metamict state. Vegard[9] thought that such minerals were probably formed under very high pressure, and as they are exposed to the ordinary pressure the crystal lattice breaks up and the change to the metamict state is reached gradually. Probably all the above factors are involved in the transformation of a mineral to the metamict state, and such a mineral is very much likely to be altered in the process of this transformation or subsequent to it by external agencies.

I would like to point out that a mineral in the metamict state is not necessarily in the altered condition. The changes to the metamict state and to the altered condition are due to two different sets of causes. The change to the metamict state signifies a change in the physical condition of the mineral and its composition is unaffected, whilst the alteration of a mineral always changes the composition of the mineral, some of its constituents being removed and replaced by other substances. The transformation to the metamict state is brought about purely by physical agencies, such as, by the radiations from the radioactive elements present in the mineral, and is also probably partly due to a release from immense pressure. These two processes may lead to an increase in volume which destroys the arrangement of atoms in the crystal lattice, thus causing the mineral to behave like an amorphous substance, *i.e.*, the mineral is then in the metamict state.

The presence of solutions is essential for altering a mineral. These solutions may contain metallic salts, silica and also some CO_2 dissolved in them. When a radioactive mineral becomes subject to the action of such solutions any of the following changes may occur: (1) The solution may exert a solvent action on some of the constituents of the mineral. (2) Chemical reactions between dissolved substances in the solution and the mineral may occur. (3) Sorption of substances in the solution by the minerals may take place. (4) Ion exchange may take place. We shall discuss these possibilities individually.

(1) There is an old observation of Brande[10] that hydrated uranium trioxide dissolves in an aqueous solution of CO_2. That this solvent action

of CO_2 is also manifest in nature is seen from the occurrence of the mineral rutherfordine, which has been found as an yellow alteration product of the uraninite of the Uruguri Mountains, East Africa.[11] The occurrence of sharpite, a radial fibrous, yellow-green carbonate from the Shinkolobwe deposit, Katanga,[12] may also be due to the same cause. Simple solution is less likely to happen than solution involving chemical reactions. It is, indeed, very difficult to distinguish between the two.

(2) That chemical reactions between substances dissolved in the percolating waters and a mineral may occur is easily understandable; here the law of mass action would decide the course of the reaction. Even very dilute solutions of acids and alkalies would cause considerable damage to a rock or mineral by prolonged action. The acid waters would easily dissolve uranium minerals, whilst alkaline waters would react with silicate and phosphate minerals, such as, allanite, monazite, etc. The presence of CO_2 dissolved in water together with salts of metals (such as those of alkalies and alkaline earths) would mostly determine the pH of the solution due to the formation of carbonates and bicarbonates. Of course, the presence of silica and other salts in the solution will cause secondary and complex reactions to take place, and of the many reaction products some will be noticeable in the alteration products of the minerals concerned and others are lost in solution.

The solvent action of acid water on uranium minerals and subsequent precipitation of uranium as phosphate is noticeable in the case of alteration of pitchblende from Gaya District, Bihar (India),[13] and also of uraninite from Ruggles mine, near Grafton Centre, New Hampshire (U.S.A.).[14] The solvent action of alkaline water has been found to be mainly responsible for the formation of thorogummite from yttrialite,[15] of tengerite from gadolinite, hellandite and yttrialite,[16] and for the alteration of allanite.[17] Further, if the percolating water is alkaline and carries silica, it is possible that when it comes in contact with the radioactive mineral, it will lose its alkalinity, and silica as such may be deposited in the mineral. This may quite happen in cases of minerals like monazite where the alkaline solution may attack the phosphate and some silica can get in. Besides these, oxidation due to oxygen dissolved in the solution and hydrolysis (in Goldschmidt's sense) may also take place. These are possibly important steps in the process of alteration of a mineral.

(3) A radioactive mineral seems to be particularly suited for sorbing substances from solution. The transformation of such a mineral to the metamict state brought about by radiations from radioactive substances and also by release from immense pressure, presumably renders the mineral somewhat porous; loss of helium from the mineral may be another cause of

its porosity. Due to the porous nature of the mineral there is greater surface, greater capillary action, Langmuir forces are more operative and the process of absorption takes place readily. Further, the sorbed salts and the compounds formed in the mineral by reaction with percolating water exert a sort of wedge action in disrupting the internal structure of the mineral; and this is one of the principal factors involved in the alteration of a mineral.

(4) The presence of UO_3 in most uranium minerals shows that a part of the uranium exists as uranate in the minerals; it may easily be conceived that part of the lead in a radioactive mineral exists as plumbate. In a mineral where uranate and plumbate ions are present, silica present in percolating waters as silicate, may easily be able to displace these ions. In uranium minerals the displacement of uranium by silica is more easily effected than that of lead because the uranate ions are present in much greater numbers than the plumbate ions. In thorium minerals where there is little uranium, lead is lost to a considerable extent. The same is also true of the thorium minerals, such as bröggerites and uranothorites, which contain comparatively large quantities of uranium. The presence of water in these minerals may be explained by supposing that hydroxyl ion replaces the acid radical.

Of the above four factors involved in the alteration of a radioactive mineral, the last one is the most important. This factor may explain why uranium minerals, suspected to be altered, tend to give higher lead ratios than pure unaltered minerals and why similar thorium minerals tend to give low results. We can also explain why the weathering of uraninite has been found to be selective in the removal of uranium,[18] and how uranium minerals of secondary origin are formed. The mode of alteration of uraninites[19] is what would be expected from the view put forward here. We have seen why thorium minerals which have undergone alteration give low age indications, and we have an explanation for Holmes' finding[20] that "the higher lead ratios of thorium minerals are, like the higher helium ratios, most likely to approach the true index to age". We may expect that thorianites being less liable to alteration through ion exchange—these being oxidic minerals—would give higher age indications than the thorites, which are likely to be altered much more easily. This has, in fact, been found to be the case by Holmes[21] in the case of minerals from Ceylon. We also understand the *raison d'etre* of the "silica test" of the freshness of titano-tantalo-niobate minerals, which Ellsworth found out as a result of his considerable experience, and why such minerals containing silica have too low lead ratios.[22]

In the alteration of phosphate minerals, like monazite, tetra-co-ordinated phosphate ion may easily be replaced by tetra-co-ordinated silicate ion.

The effect of alteration on the lead ratios of monazite and similar minerals would be expected to lower the lead ratios according to the ideas put forward in this paper. Such will indeed be the case if the mineral is considerably altered. When the alteration is slight, the silicate ion will mostly replace the phosphate ion, while the plumbate ion, being much less in number, will not be replaced. From this reasoning, the age indications of slightly altered monazites may be reliable.

In the process of alteration due to ion exchange, both the cations and anions are involved. Ion exchange involving cations does not seem to be of much importance as regards the removal of lead, uranium or thorium, though the possibility of the removal of thorium and uranium by calcium and that of lead by potassium is not denied; but it is thought that such exchange would be important for indicating that the mineral has been subjected to the action of percolating water. Alkalies are usually not present in such minerals, and their presence would indicate alteration of the mineral. It has been observed that in secondary uranium minerals, silication and hydration are quite common; alkalies have also been found when sought for. Generally speaking, in pitchblendes, an increased silica content is accompanied by hydration, and alkali salts are usually present in such cases. The hydration is not always in evidence; when the alteration is not serious alkalies are absent, silica and water are low and one of these may be very low or absent altogether. The presence of alkalies in addition to silica and water in relatively large quantities is a sure indication that the mineral has been considerably altered. In thorianites—these being oxidic minerals—ion exchange is not possible and consequently we find little water, practically no silica and no alkalies at all; in contrast to these, thorites often contain large amounts of water (6–10 per cent.) and appreciable amounts of alkalies (0·25–0·82 per cent.). Uranothorites also contain water (9·3–12·0 per cent.) and alkalies (0·11–0·77 per cent.). The decomposition products of thorites, *e.g.*, calciothorites, freyalite eukrasite and mackintoshite contain appreciable quantities of alkalies (0·7–2·5 per cent.). The same is true of the zirconium minerals and the titano-tantalo-columbates.[23]

In the case of monazites it is rather difficult to ascertain from analytical data whether they have undergone any alteration. It is not justifiable to think that the silica is present in monazite as thorite,[24] and the absence of thorite from monazite (in thin sections) does not mean that the presence of silica is a result of alteration. In monazite silica is as much a primary constituent as is phosphate. Water is almost always found; Johnstone[25] observed that combined water in monazites varied from 0·2 to 1·2 per cent.

In such a case the presence of alkali salts in the mineral may be considered as an indication of the altered nature of the mineral. I want to emphasise the point that the presence of small quantities of silica and water in a mineral is no indication of its alteration. The age calculated from such minerals may be reliable. When comparatively large quantities of silica and/or water are present, the mineral may be considered to have undergone alteration; in all such cases alkalies will be found to be present in the minerals. The presence of alkalies is the only sure criterion by which a suspected mineral normally containing silica and water may be judged to have undergone alteration.

It is to be noted that our ideas as regards the process or mechanism of alteration is a general one and applicable not only in the case of the radioactive minerals but in the case of other minerals also. To give an example: all titanium minerals which contain appreciable quantities of water and silica contain alkalies[23]; these minerals are to be regarded as altered.

It would thus seem that from a chemical analysis silica, water and alkali content of a mineral would be a fair indication whether its lead ratio would be reliable or not. The relative abundance of UO_3 over UO_2 in a mineral is due to auto-oxidation; and it would be rash to consider it as indicative of the altered nature of a mineral. The preponderance of UO_3 over UO_2 may be caused by alteration, but is not necessarily a sign of it.

Holmes[21] in 1926 suggested " that the discrepancies in thorium minerals are due to the fact that lead present as oxide or silicate would be more easily removed by percolating waters and kinetic exchange than the lead in uranium minerals, which may be present actually or potentially as a highly insoluble uranate". This was as a result of the study of the lead ratios obtained from many thorium minerals and a smaller number of uranium minerals from Norway, Ceylon and U.S.A., and based on the atomic weight of the lead and the amounts of uranium and thorium present in the mineral as found by analysis. He calculated the amounts of thoirum-lead and uranium-lead and found that the age as deduced from the accumulation of thorium-lead is, in practically all cases, lower than that deduced from the accumulation of uranium-lead. Almost all the minerals examined by Holmes were altered specimens. In short, Holmes' selective leaching hypothesis recognises that the minerals concerned have undergone leaching and thorium-lead (also to a much less extent uranium-lead) has been leached out as a consequence.

He later on recognises[26] that " the hypothesis cannot, of course, represent the whole truth, for it is not to be supposed that while lead was being leached out of a mineral the proportion of uranium and thorium would continue to remain unchanged". Herein lies the greatest weakness in Holmes' theory

and the chief difficulty in our accepting it. Holmes' hypothesis is based on results deduced from admittedly altered minerals and cannot find acceptance. Gleditsch and Quiller's[27] corroboration of Holmes' idea is not tenable as their investigation was carried out with quite altered minerals. R. C. Wells[28] has adversely criticised Holmes' idea as it postulated different behaviours of the isotopes of the same element, namely, uranium-lead and thorium-lead, towards circulating water, and has suggested, following Ellsworth, the alternative explanation that uranium has been leached out relative to other elements. It also follows that Holmes' idea that[29] " when lead is lost it provides solutions that are relatively rich in thorium-lead, and when lead is gained the latter presumably derived from the same solutions—is also relatively rich in thorium-lead " becomes unacceptable.

The irregularities in lead ratios shown by different minerals from the same locality may actually indicate different periods of their formation, as has been thought by Hess[30] and others and for which Hess and Wells[31] seem to find a support. Following the same current of idea, Kirsch[32] suggested rejuvenation of magma as explaining the erratic lead ratios of the bröggerites from Moss, Norway. Holmes' comment[33] on the idea is " that a very top-heavy superstructure has been raised on extremely slender and inadequate supports," but he admits of the possibility of there being two periods of bröggerite formation. He further says[34] " that the lead ratios of certain other suites of minerals also suggest that the latter were the products of two periods of igneous activity rather than one ; such are the uraninites of Morogoro with ratios 0·084 and 0·091 and possibly the thorianites and uraninites of Ceylon with respective ratios of Ca 0·08 and 0·065".

Kirsch in collecting his data of the lead ratios of the bröggerites from Moss, Norway, included the lead ratios of altered minerals and of those which had not been analysed sufficiently accurately for the purpose. The inclusion of such lead ratios invalidates any theory based on such data. That the minerals of a pegmatite may be changed long after its formation by later igneous activity in the same place is a possibility that is not denied, but irregular lead ratios shown by radioactive minerals of the same pegmatite is not necessarily a *proof* of such rejuvenation of magma. In my opinion Kirsch's idea as regards Moss bröggerites is not tenable and even Holmes' modification of the same idea is not warranted by the existing data.

The reasons for assuming that common lead is present in radioactive minerals seem to be the following: (1) Kirsch[35] found that the atomic weight of lead derived from some radioactive minerals differed from that calculated from the uranium and thorium content of the minerals, and he ascribed this

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difference to the presence of common lead in the minerals. (2) Kovarik[36] argued that since Joachimsthal pitchblende contained common lead, other radioactive minerals would similarly contain it to some extent. (3) He tried to support his argument with the observation that the ratio $\lambda_u : \lambda_{Th}$ calculated from different radioactive minerals of different geological ages, was not a constant as it ought to be.

Ellsworth[37] as well as Holmes,[21] on mineralogical evidence, do not entertain the idea that common lead occurs in radioactive minerals derived from pegmatites. Ellsworth also points out that Kirsch's finding may be due to the altered nature of the minerals examined. Kovarik's first argument is not tenable, because the amorphous nature of the Joachimsthal pitchblende, its mode of occurrence and the associated minerals, all go to make its case a special one, and not representative of the radioactive minerals derived from pegmatites. His second argument would have been valid if the minerals he examined were perfectly fresh and unaltered, which they were not. In such minerals naturally the ratio $\lambda_u : \lambda_{Th}$ which is equal to $\frac{Th}{U} \cdot \frac{206}{208} \cdot \frac{208-A}{A-206}$ would not be a constant. Incidentally the reason why Kirsch's[38] method of determining λ_{Th} gave an incorrect value lies in the fact that the Ceylon thorianite, which Kirsch utilised, was not quite fresh and consequently gave unreliable data.[39]

We may then accept that no common lead is present in radioactive minerals derived from pegmatites, and it is therefore unnecessary to determine the atomic weight of lead from a mineral. Such a determination would only indicate whether a mineral is altered or not. The altered character of a mineral can easily and definitely be known from physical and chemical examinations, without taking the trouble of an atomic weight determination.

Summary

The discordant lead ratios of uranium and thorium minerals gave rise to a number of unsuccessful but interesting ideas to explain them. A fresh attempt has been made here based on the alteration of radioactive minerals. The alteration of a radioactive mineral is distinguished from its transformation to the metamict state. The latter is entirely caused by physical agencies, whereas the presence of solution is essential for altering a mineral. Such a solution may exert (1) a solvent action; or (2) chemically react with a mineral; (3) sorption of substance from the solution as well as (4) ion exchange may take place. Each of these possibilities has been considered individually.

In (4) the supposition is made that part of lead in the radioactive minerals is present as plumbate just as uranium is partly present as uranate.

Silica present in percolating waters as silicate may displace plumbate and uranate ions. This idea successfully explains (1) the high lead ratio obtained from altered uranium minerals, (2) low lead ratios from all altered minerals containing small quantities of uranium, (3) the alteration of uraninites, and (4) the *raison d'etre* of the " silica test " for the freshness of titano-tantalo-columbates.

Reasons are given for the belief that age indications of slightly altered monazites may be reliable, that the presence of alkalies in a primary radioactive mineral is a sign of its alteration, and that the process of alteration as considered here is a general one and applicable to non-radioactive minerals as well.

Holmes' selective leaching hypothesis, Kirsch's idea of magmatic rejuvenation to explain erratic lead ratios of Moss bröggerites and the idea of possible presence of common lead in radioactive minerals have been discussed and found unacceptable.

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AMMONITES FROM THE BAGH BEDS

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1. Introduction

Ammonites as compared to the other groups of fossils, though well preserved, are very poorly represented in this series of strata, both in variety and number. Identifiable specimens were first collected by P. N. Bose[1] and assigned byFeistmantel[2] to *Ammonites guadaloupae* Roemer from the Upper Cretaceous of Texas.[3] A closer study by Vredenburg,[4] however, revealed among these the presence of three species—*Placenticeras mintoi* Vredenburg, *Namadoceras scindiæ* Vredenburg and *N. bosei* Vredenburg. Of these the first species was considered by him to belong to the "Group of *Placenticeras fritschi*" ranging from the Gault to the Senonian, thus roughly corroborating Duncan's view about the Cenomanian age of the Bagh Beds.[5] Fourteau[6] while discussing the age of these beds on the basis of his study of the echinoids remarks that *Placenticeras mintoi* Vred. is closely allied to *Pl. saadense* Peron and Thomas[7] from the Vraconian of Algeria and Tunis and to *Pl. uhligi* Choffat[8] from the Bellasian of Portugal, thus pointing to Gault as the more probable age. Dr. Spath,[9] however, while discussing the age of the Samana Range Cephalopoda, makes a stray remark that the Bagh ammonites are Turonian, in any case younger than Cenomanian.

2. Palæontological Remarks

Knemiceras mintoi (Vredenbrug)

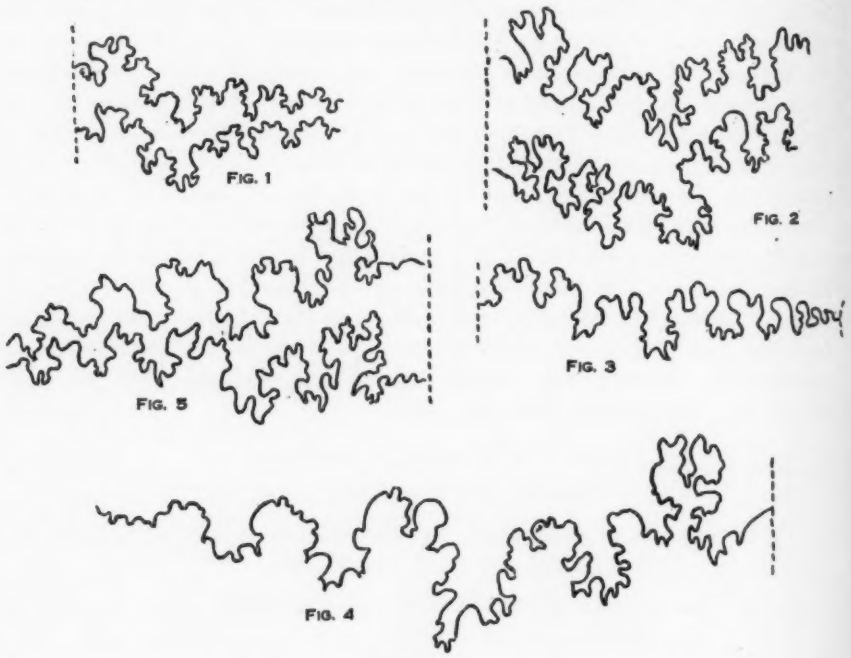
1884. *Ammonites guadaloupae* Feistmantel (non Roemer); Bose, *Mem. G.S.I.*, Vol. 21, Pt. I, pp. 37, 40.

1907. *Placenticeras mintoi* Vredenburg, *Rec. G.S.I.*, Vol. 36, p. 111, Pls. 14, 15.

The material representing this species consists of a number of well preserved specimens, young and adult, the largest which is incomplete, in

[1] Vredenburg, 1907, 110.
[2] Bose, 1884, 37, 40; Oldham, 1893 250.
[3] Roemer, 1852, 32; Hyatt, 1903, 197.
[4] Vredenburg, 1907, 109–25.
[5] Vredenburg, 1907, 111.
[6] Fourteau, 1918, 53.
[7] Peron and Thomas, 1889, 19.
[8] Choffat, 1898, 4, 77; Hyatt, 1903, 152. Pervinquière, 1910, 39.
[9] Spath, 1930, 64.

the author's collection measuring 110 mm. in diameter; the different stages of growth of the suture line and the whorl-section can thus be studied. Though there is as remarked by Vredenburg, some amount of variation in the adult ventre, which is at times more or less rounded, on the whole they have at younger stages a trapezoidal whorl-section; while, the suture line reproduced here from Vredenburg's paper (Figs. 1, 2) clearly consists of three derived lobules in the first lateral lobe. These characters clearly indicate that this form is a species of *Knemiceras*.[10] Vredenburg while recognising the near relation between the suture line of the present species and that of *Kn. saadense* (Peron and Thomas) from the Vraconian of Algeria and Tunis and of *Kn. ebrayi* (de Loriol[11]) from the lower Gault of Switzerland,[12] was



FIGS. 1–5

FIGS. 1–2.—Suture lines of *Knemiceras minoti*. FIG. 3. Suture line of *Kn. ebrayi*. FIG. 4. Suture line of *Namadoceras scindiæ*. Fig. 5. Suture line of *N. bosei*.

[10] Böhm, 1889, 198–200. Pervinquière, 1910; 32.

[11] With this species as the type, Spath (1924, 508) creates a new genus *Parengonoceras*, because of its suture line far less Ceratitic than that of *Engonoceras*, *Protengonoceras* and *Metengonoceras*, and different from *Hypengonoceras*. But in the absence of sufficiently detailed study to justify the creation of a new genus, the present writer cannot accept this new generic name.

[12] Loriol, 1882, 1; Douvillé, 1911, 317, Fig. 62; Hyatt, 1903, 234; Vredenburg, 1907, 119.

inclined to consider it to have its nearest ally in *Kn. tamulicum* (Kossmat) from the Trichinopoly stage (lower Senonian) of the South Indian Cretaceous Series.[13] A careful comparison of the suture line of the present species with that of *Kn. ebrayi* (de Loriol) (Fig. 3) reveals that the adults of the former show a stage of development later than that seen in the latter species; similar relations obtain between the present species and *Kn. uhligi* (Choffat) from the Bellasian (upper Gault to lower Cenomanian) of Portugal, thus clearly showing that *Kn. mintoi* is a little newer than, and also stands closest to these Swiss and Portuguese species.

Genus *Namadoceras* Vredenburg, 1907

This genus was created by Vredenburg to include two sphenodiscoid ammonites having a broad external saddle divided by deep lobules into two main portions, and followed by a moderate development of lateral and auxiliary series. He[14] discusses its relation to different genera, such as *Placenticeras*, *Indoceras*, *Sphenodiscus*, etc., and admits the possibility of its being a veritable *Coelopoceras*.[15]

Douvillé[16] also points out the close similarity between the general aspect of the suture lines of *Namadoceras scindiæ* (Fig. 4) and *N. bosei* (Fig. 5) with that of *Coelopoceras requieni* (Fig. 6) and *Coel.* cf. *requieni* (Fig. 7) respectively, and proposes the relegation of this genus to the synonymy of *Coelopocer*.

The only two known species of *Namadoceras* are represented, each by a single adult specimen. It has therefore, not been possible to study the developmental stages of their sutures nor to verify if they develop sub-acute whorls directly on the rounded ventre of nepionic stage, and have a hollow keel[17] as in the genus *Coelopoceras*.

Here a reference must also be made to the close resemblance which obtains between the suture line of *Namadoceras bosei* and *Hoplitoides* cf. *ingens nodifer*[18] (Fig. 8).

Admitting thus the possibility of *Namadoceras*, being synonymous with *Coelopoceras* as suggested by Douvillé and Vredenburg, in the absence of more definite information based on the study of larger number of specimens,

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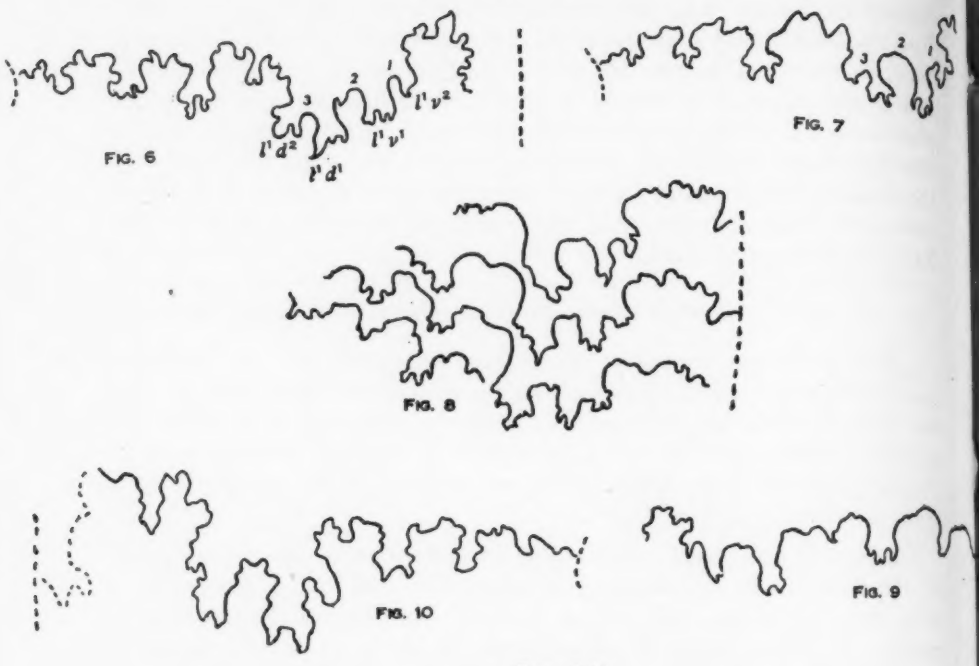
[13] Stoliczka, 1866, 90; Kossmat, 1895, 174; Hyatt, 1903, 241; Vredenburg, 1907, 111–14.
[14] Vredenburg, 1907, 121–22; 1908, 239–40.
[15] Hyatt, 1903, 88–91; Pervinquière, 1907, 222, 386; 1910, 71–76. Vredenburg, 1908, 239–40.
[16] Douvillé, 1911, 288, 309, Figs. 36, 37.
[17] According to Pervinquière (1910, 72–76) hollow keel is not a constant feature of this genus.
[18] Douvillé, 1928, 29, Fig. 20.



FIGS. 6–10.

FIG. 6. Suture line of *Coelopoceras requieni*. FIG. 7. Suture line of *Coel. cf. requieni*. FIG. 8. Suture line of *Hoplitoides cf. ingens nodifer*. FIG. 9. Suture line of *Coelopoceras lesseli*. FIG. 10. Suture line of *Coel. africanum*.

it is desirable that *Namadoceras*, be for the time being, retained as a genus distinct from *Coelopoceras* to which it appears to be very closely related.

3. *Age of the Bagh Ammonites*

It is rather unfortunate that ammonites which are always of great help in correlation of formations in widely separated areas, are so scantily represented among the Bagh fauna. Of the three species known to occur in these beds *Knemiceras mintoi* (Vred.) as mentioned above has newer appearance than its nearest allies *Kn. ebrayi* from the Gault of Switzerland and *Kn. uhligi* from the Bellasian of Portugal.

Namadoceras scindiæ and *N. bosei* as pointed out by Douvillé, have the general aspect of their suture lines very much like those of *Coelopoceras requieni* and *Coel. cf. requieni* from the upper and lower Turonian respectively, of Khenchela. But a closer examination will reveal that in *Namadoceras* the lobules (*e.g.*, 1^1d^1 and 1^1d^2) have not yet as much individualised as in

Coelopoceras requieni and *Coel. cf. requieni*. As regards the stage of subdivision of the lateral lobe, a closer resemblance is shown by *Namadoceras scindiæ* to the Turonian form *Coel. lesseli*[19] (Fig. 9), while *N. bosei* shows the stage of development of the suture line which is found to have been attained in *Coel. africanum*[20] (Fig. 10) from the middle Cenomanian of Algeria. Thus *Namadoceras*, though it admittedly holds an insecure generic position, shows distinctly a range of affinities from middle Cenomanian to Turonian.

The unsubstantiated statement by Spath that the Bagh ammonites are Turonian and certainly younger than Cenomanian is already referred to in the Introduction. Dr. Spath in the course of personal correspondnece[21] in this connection, through Dr. Raj Nath, re-affirms this statement by saying that though he could not point to any published reference, he, while expressing the above opinion in the year 1930, perhaps had the recollection of having discussed *Namadoceras* with Vredenburg.

Since none of the three Bagh ammonite species is known to occur elsewhere in deposits of undisputable age, it is not possible to fix the age of the Bagh ammonite fauna with any great precision. In the light of the foregoing discussion on their affinities towards foreign species, upon which entirely we have to depend, the writer does not see his way to accepting Spath's *verdict*, as he calls it, but finds it more reasonable to regard middle to upper Cenomanian as a very probable age of these ammonites.

Acknowledgments

In conclusion, the author has to sincerely thank Dr. Raj Nath for kindly going through the manuscript of this paper and for his valuable criticism. Thanks are also due to Mr. V. P. Sondhi for kindly verifying some of the references, and to the Director of the Geological Survey of India for the permission to work in the Survey Museum and Library. The author also takes this opportunity of expressing his indebtedness to the Dhar, Indore and Gwalior Durbars for the kind permission to collect fossils from within their territories and for the facilities given during the field work.

[19] Douvillé, 1911, 309, Fig. 39.
[20] Pervinquière, 1910, 73, Douvillé, 1911, 308, Fig. 35.
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AN ANALYSIS OF TWO HUNDRED NORMAL ELECTROCARDIOGRAMS

(Subjects Aged 11 to 50 Years)

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The present study was undertaken with a view to determine normal electrocardiographic standards for Indian subjects. Standards in use in India, at the present day, being unfortunately based on small series of European or American subjects, are inadequate for our needs; they are not in keeping with the growing interest of the clinician in the subject of Electrocardiography. The correct interpretation of an Electrocardiogram demands, first and foremost, a thorough knowledge of the normal state, with all its physiological variations and limits. A search of the literature has failed to reveal a single large-scale investigation carried out in this country into the subject of normal electrocardiographic standards. In two previous issues of these *Proceedings* I have presented detailed studies of normal electrocardiographic records from school-children, aged 5 to 15 years. In the present issue, the study has been extended to a further series of 200 normal subjects, in the 2nd, 3rd, 4th and 5th decades of life. For the sake of uniformity, twenty-five male and twenty-five female subjects have been allotted to each of these four decades, making a total of one hundred male and one hundred female subjects.

An attempt has been made to determine the limits of normal or physiological variation of the various ingredients of the Electrocardiogram, in different age and sex groups.

Selection of Material

Subjects were chosen from diverse sources. Some were medical students, some were friends and relatives who kindly volunteered to their being electrocardiographed, the rest being private and hospital patients with minor surgical and non-cardiac complaints.

Each subject was subjected to a thorough clinical examination in order to exclude the presence of any cardio-vascular disease or any disease capable

of influencing to the electrocardiogram. All cases with symptoms or signs even remotely suggestive of cardiac involvement, *e.g.*, hæmic murmurs, were rigidly excluded from the present investigation.

The tracings were recorded on a Victor (G.E.C.) portable electrocardiograph, with the subject at rest and in a sitting position. Measurements were made in accordance with the standard technique.

The P-Wave

This wave represents early activity of the auricular muscle-fibres; it ends sometime before the actual completion of auricular systole.

Form of the P Wave.—The P wave, which is usually described in text-books as a smooth rounded elevation, actually displays a great variety of forms; it may display rounded, pointed, flat, bifid, inverted and diphasic forms. It has already been demonstrated that the pointed form of P is much commoner than the rounded form in children of school-age. This was found to be the case in the present series of adults also; the percentage frequencies of the pointed and rounded forms of P in this series were 46% and 35% respectively.

The higher incidence of the pointed form of P was observed in all the three standard leads of the Electrocardiogram. The *pointed* form of P was observed in Lead I in 48% of records, in Lead II in 52·5% and in Lead III in 36%. The *rounded* form was observed in Lead I in 39·5%, in Lead II in 36·5% and in Lead III in 29%. Pointed and rounded forms of P were encountered in 6·5% of records in Lead I, in 4·5% in Lead II and in 5·5% in Lead III. *Flat* forms of P were observed in Lead I in 2·5%, in Lead II in 2·0% and in Lead III in 6·5% of records, while the *bifid* form of P occurred in 1·5% records in Lead I, 2·5% in Lead II and 2·0% in Lead III. The *inverted* form of P wave was noted in Lead I in 0·5% records, in Lead II in 1·0% and in Lead III in as many as 12·5% records. The *diphasic* form of P occurred in 1·5% records in Lead I, in 1·0% records in Lead II and in 9·5% records in Lead III.

P waves, intermediate in form between the pointed and the rounded form of P and also, P waves varying in form from the pointed to the rounded or *vice versa*, were classed together in the group known as the "pointed and rounded form" of P wave.

Notching of P Waves.—Though formerly recognised as a reliable sign of auricular muscle hypertrophy, P wave notching has now lost its previous significance, having been repeatedly observed by various observers in perfectly normal records.

In my series of normals, notching of the P wave was observed quite frequently. Taking all records into consideration, notching of P was observed in one or more leads in as many as 77 records (*i.e.*, 38·5%); 34 records were from male subjects and 43 from females.

In only 5 cases was notching of P observed in all the three standard leads. Notching of P_1 was noted in 37 records (18·5%), of P_2 in 49 records (24·5%) and of P in 41 records (20·5%). In 40 records (20%), there was notching of P_3 in two leads; notching of P_1 and P_2 in 16 records, of P_2 and P_3 in 16 records and of P_1 and P_3 in 8 records. In 32 records (16%), there was notching of P confined to one lead only; notching of P_1 in 8 records, of P_2 in 12 records and of P_3 in 12 records.

In the 2nd decade group, notching of P was observed in 44% of records (40% of males and 48% of females). In the 3rd decade, notching occurred in 34 % of records (32% of male and 36% of female records). In the 4th decade, notching of P was noted in 34% of records (28% of male and 40% of female records). In the 5th decade, notching was observed in 42% of records (36% of males and 48% of females). It will be noted from the above data, that notching of P is rather more common in normal female than in normal male subjects (ratio of 4 : 3). The incidence of P wave notching was somewhat higher in the 2nd and 5th decades than in the 3rd and 4th decades, in the present series.

Notching in P Waves of Large Amplitude.—It has repeatedly been asserted in medical circles that a combination of notching and large amplitude (over 2 mm.) of the P wave cannot be considered a normal variation and should suggest a diagnosis of mitral stenosis. In the present series of 200 normal records, there were 6 records with P waves notched and more than 2 mm. in amplitude; the amplitudes of P waves in these cases were 2·3 mm., 2·4 mm., 2·1 mm., 2·6 mm., 2·2 mm., and 2·4 mm., respectively; there was no evidence of mitral disease in any of these 6 cases. On the basis of this new series of 200 cases, I can reaffirm my previous statement that in a small percentage of perfectly normal records (3%) there may be an association of notching and large amplitude of the P waves.

Relation of T to P Waves in Lead III.—A close relationship or association was observed between the P and the T waves by Hoskin and Jonescu and by Shipley and Hallaran. Inverted P waves meant inverted T waves and diphasic P waves meant diphasic T waves. A study of the present series of normal records supports the first half of this contention. Inverted P waves were observed in Lead III in 12·5% of my records; in every single case, the corresponding T wave was also inverted. Inversion of P_3 was commoner in

females than in males and reached a high incidence in the 5th decade; an incidence rate of 28% in female subjects of the 5th decade.

With regard to diphasic P waves, my findings are not in conformity with those of the above authors. Diphasic P waves in Lead III were observed in 9·5% of my cases, *i.e.*, in 19 records. Of these 19 records, 10 showed inverted T_3 waves, 4 (21%) showed diphasic T_3, 3 (16%) showed flat T_3 waves and 2 records showed upright T_3 waves. On considering diphasic P_3 waves, the corresponding T deflections were found to display no constant shape or direction.

Flat P_3 waves were noted in 13 records (*i.e.*, 6·5%); of these, 7 records showed flat T waves, 4 showed inverted T waves and 2 showed upright T waves.

Amplitude or Height of the P Waves.—The amplitude of P was found to vary from 0·5 mm. to 5·0 mm. with an average value of 1·74 mm. by Hoskin and Jonescu, in their series of fifty normal women. According to Lewis and Gilder, the average values of P for the three standard leads are 0·52 mm., 1·16 mm., and 0·81 mm., respectively.

In my series of 200 normal subjects, the average value of P, taking the three standard leads into consideration, was 1·00 mm. The average value for Lead I was 0·89 mm., for Lead II 1·35 mm., and for Lead III 0·76 mm. High values for P were most often encountered in Lead II, as shown by previous workers, though the maximum value of 3·2 mm. for the whole series was encountered in Lead III.

Range.—In Lead I the height of P ranged from − 0·3 to 2·3 mm. in Lead II from − 0·2 to 3·1 mm.; in Lead III from − 2·0 to 3·2 mm. (Please see Table III.)

2nd Decade.—The average value of P for male subjects was 0·90 mm. with a range of − 1·1 to 3·0 mm. For females, the average was 0·89 mm., and the range − 1·0 to 3·2 mm.

3rd Decade.—The average for males was 0·94 mm., and the range − 2·0 to 3·1 mm.; the average for females was 0·86 mm., and the range − 1·3 to 3·0 mm.

4th Decade.—The average value for males was 1·25 mm., and the range − 0·5 to 3·1 mm.; for females, the average was 0·96 and the range − 0·2 to 2·1 mm.

5th Decade.—For males, the average value was 1·05 mm., and the range, − 1·2 to 2·3 mm.; for females, the average was 1·05 mm. and the range, − 1·0 to 2·2 mm.

The value of P wave amplitude was somewhat higher in males than in females, in all the standard leads. This is in conformity with the analysis of Shipley and Hallaran.

The average values for P were somewhat higher in the 4th and 5th than in the 2nd and 3rd decades of life.

(For further particulars on P wave amplitude see Table IV.)

Duration of P Wave.—In my series, the mean value of P wave duration for the three standard leads was 0·083 sec. The average value for Lead I was 0·080 sec., for Lead II 0·092 sec., and for Lead III 0·077 sec. High values for P wave duration were most frequently encountered in Lead II.

Range.—In Lead I, the duration of P ranged from 0·04 to 0·15 sec.; in Lead II, from 0·03 to 0·15 sec.; in Lead III, from 0·03 to 0·14 sec. (See Table V.)

2nd Decade.—The average value of P duration for males was 0·080 sec., with a range of 0·04 to 0·15 sec.; for females, the average was 0·080 sec., and the range 0·03 to 0·14 sec.

3rd Decade.—For males, the average was 0·085 sec., and the range 0·03 to 0·15 sec.; for females, the average was 0·082 sec., and the range 0·04 to 0·14 sec.

4th Decade.—For males, the average was 0·087 sec., and the range 0·04 to 0·14 sec.; for females, the average was 0·083 sec., and the range 0·03 to 0·15 sec.

5th Decade.—For males, the average was 0·083 sec., and the range 0·04 to 0·15 sec.; for females, the average was 0·080 sec., and the range 0·04 to 0·14 sec.

P Wave duration appeared to be somewhat higher in males than in females. The average value of P duration shows little or no alteration with age.

P–Q Duration

This is measured from the end of P to the beginning of the Q wave.

In my series, the average value of P–Q duration, taking all leads into consideration, was 0·056 sec. The average values for the individual leads were as follows: Lead I = 0·052 sec.; Lead II = 0·056 sec.; Lead III = 0·061 sec.

Range.—In Lead I, P–Q duration varied from 0·01 to 0·12 sec., in Lead II from 0·00 to 1·12 sec., and in Lead III from 0·01 to 0·13 sec. (See Table VI.)

Maximum values for P–Q duration were observed in Lead I 24 times (12%), in Lead II 44 times (22%) and in Lead III 64 times (32%). A P-Q duration constant in the three leads was noted in 6 records (3%). Maximum values for P–Q were observed in two leads in 22 records (11%), 6 times in Leads I and II, 12 times in Leads II and III and 4 times in Leads I and III.

2*nd Decade*.—The average value of P–Q for males was 0·061 sec., with a range of 0·01 to 0·12 sec.; for females, the average was 0·047 sec., and the range of 0·00 to 0·10 sec.

3*rd Decade*.—The average value for males was 0·059 sec., with a range of 0·02 to 0·12 sec.; for females, the average was 0·050 sec., and the range 0·02 to 0·12 sec.

4*th Decade*.—The average value for males was 0·062 sec., with a range of 0·01 to 0·13 sec.; for females, the average was 0·059 sec., and the range 0·01 to 0·10 sec.

5*th Decade*.—The average value for males was 0·058 sec., with a range of 0·01 to 0·12 sec. ; for females, the average was 0·052 sec., and the range 0·01 to 0·12 sec.

The average values for P–Q duration were higher in males than in females, in the present series. Average values were higher in Lead III than in either Lead I or II.

P–R Interval

This is measured from the beginning of the P wave to the beginning of the QRS Complex. It represents the auriculo-ventricular conduction time.

The average value for P–R duration (in the present series), taking all leads into consideration was 0·148 sec. The average value for Lead I was 0·141 sec., for Lead II 0·149 sec., and for Lead III 0·154 sec.

Range.—In Lead I, the P–R interval ranged from 0·08 to 0·21 sec., in Lead II from 0·07 to 0·21 sec., and in Lead III from 0·07 to 0·21 sec. (See Table VII.)

2*nd Decade*.—The average value of P–R for male subjects was 0·143 sec., with a range of 0·07 to 0·21 sec.; for females, the average was 0·123 sec., and the range 0·08 to 0·20 sec.

3*rd Decade*.—For males, the average was 0·147 sec., and the range 0·10 to 0·20 sec.; for females, the average was 0·138 sec., and the range 0·09 to 0·21 sec.

4*th Decade*.—For males, the average was 0·165 sec., and the range 0·11 to 0·21 sec.; for females, the average was 0·155 sec., and the range 0·09 to 0·20 sec.

5th Decade.—For males, the average was 0·157 sec., and the range 0·10 to 0·21 sec.; for females, the average was 0·154 sec. and the range 0·09 to 0·21 sec.

The average values for the P–R interval were higher in males than in females and higher in the 4th and 5th than in the 2nd and 3rd decades. P–R values were found to be higher in adults than in children.

P–R Interval in the Lead of Largest Measurements.—The two hundred records of my series were investigated from this point of view. Maximum P–R values were observed 72 times (36%) in Lead I, 120 times (60%) in Lead II and 100 times (50%) in Lead III. The P–R interval was identical in the three leads in 16 records (8%). Maximum values for P–R were shared by two leads on 60 occasions (30%), 16 times in Leads I and II, 36 times in Leads II and III and 8 times in Leads I and III.

Values for the maximum P–R duration ranged from 0·09 to 0·21 sec., in the present series, the average value being 0·162 sec. For further details, please refer to Table VIII.

P–R Level or the Auricular T Wave

The P–R level is seldom iso-electric in all the three leads. There is usually some degree of deflection or deviation from the Zero-line, practically always in a downward direction.

In my series of 200 normal records, a downward deflection of P–R was observed in all three leads in 77 records (38·5%) and in two leads in 76 records (38%); deviation of P–R confined to one lead only was observed in 17 records (8·5%). In 30 records (15%) there was no deflection of the P–R level in any lead. (For detials, see Table IX.)

Measurement of P–R Level Deflection.—This is measured from the Zero-line, base-line or iso-electric line of the record.

In my series, the average value of the deflection, taking all leads into consideration, was 0·31 mm. The average values for the individual leads were 0·28 mm. for Lead I, 0·36 mm. for Lead II, and 0·30 mm. for Lead III.

Range.—In Lead I, the deflection of P–R ranged from 0·5 to 2·0 mm., in Lead II from 0 to 2·2 mm., and in Lead III from 0 to 2·1 mm. (See Table X.)

2nd Decade.—The average value of P–R deflection was 0·28 mm. for males, with a range of 0 to 2·0 mm.; for females, the average was 0·27 mm. and the range 0 to 2·1 mm.

3rd Decade.—For males, the average was 0·29 mm. and the range 0 to 2·0 mm.; for females, the average was 0·30 mm., and the range 0·0 to 1·8 mm.

4th Decade.—For males, the average was 0·34 mm. and the range 0 to 2·0 mm.; for females, the average was 0·33 mm. and the range 0 to 2·0 mm.

5th Decade.—For males, the average value was 0·35 mm. and the range 0 to 2·0 mm.; for females, the average was 0·33 mm. and the range 0 to 2·2 mm.

The average values for P–R level deflection were not markedly different in the two sexes, though the values tended to be higher in males in Leads I and II and in females in Lead III. Average values were higher in Lead II than in either Lead I or III.

QRS Complex or Group

Duration of QRS Complex.—It is measured from the beginning of the initial ventricular deflection to the end of the final deflection of QRS.

In my series of 200 normal records from adults, the average value of the QRS duration for all leads was 0·073 sec. The average values for the individual leads were Lead I = 0·073 sec. ; Lead II = 0·072 sec.; Lead III = 0·073 sec.

Range.—In Lead I, the QRS duration ranged from 0·03 to 0·10 sec., in Lead II from 0·03 to 0·11 sec., and in Lead III from 0·03 to 0·11 sec. (See Table XI.)

2nd Decade.—The average value for QRS duration was 0·068 sec. for males, with a range of 0·03 to 0·11 sec.; for females, the average was 0·067 sec., and the range 0·03 to 0·11 sec.

3rd Decade.—The average for males was 0·070 sec., with a range of 0·05 to 0·11 sec.; for females, the average was 0·068 sec., and the range 0·04 to 0·10 sec.

4th Decade.—For males, the average was 0·076 sec. and the range 0·04 to 0·11 sec.; for females, the average was 0·077 sec., and the range 0·06 to 0·10 sec.

5th Decade.—For males, the average was 0·079 sec., and the range 0·04 to 0·11 sec. ; for females, the average was 0·075 sec., and the range 0·05 to 0·10 sec.

The average values for QRS were higher in adults than in children (0·073 sec. as compared to 0·067 sec. for children of school-age) and

higher in males than in females (for males 0·073 sec.; for females, 0·072 sec.). The duration of QRS was practically constant in the three leads. The shorter duration of QRS in children is probably due to a shorter conduction system and a smaller ventricular mass.

Duration of QRS in the Lead of Largest Measurement.—The two hundred records of my series were investigated from this point of view. Maximum values of QRS duration were observed 84 times (42%) in Lead I, 96 times (48%) in Lead II and 80 times (40%) in Lead III. In 15 records (7·5%), the QRS duration was constant in all the three leads. Maximum values for QRS duration were shared by two leads on 29 occasions (14·5%), 12 times in Leads I and II, 9 times in Leads II and III and 8 times in Leads I and III. Values for maximum QRS duration ranged from 0·05 sec. to 0·11 sec., in the 200 records, with an average value of 0·080 sec. (See Table XII for further details.)

The Q Wave or Deflection

Incidence of the Q Wave.—In my series of records (all cases included) a Q wave was observed in Lead I in 51% of cases, in Lead II in 58% and in Lead III in 66%. In other words, in a series of 200 records, the Q wave was absent in Lead I on 98 occasions, in Lead II on 84 occasions and in Lead III on 68 occasions. A Q wave was observed in all the three leads in 44 records (22%). In 76 records (38%) a Q deflection was noted in two leads, 17 times in Leads I and II, 42 times in Leads II and III and 17 times in Leads I and III.

In males, a Q deflection was observed in Lead I in 53% of records, in Lead II in 58% and in Lead III in 70%.

In females, A Q wave was present in Lead I in 49%, in Lead II in 58% and in Lead III in 62% of records. (See Table XIII.)

Amplitude of Q Wave.—In my series, the average value of the Q wave amplitude, taking all leads into consideration, was 0·48 mm. The average value for Lead I was 0·35 mm., for Lead II 0·47 mm., and for Lead III 0·63 mm.

High values for Q were encountered most frequently in Lead III.

Range.—In Lead I, the amplitude of Q ranged from 0 to 4·1 mm.; In Lead II from 0 to 3·0 mm. and in Lead III from 0 to 6·0. (See Table XIV.)

2*nd Decade.*—For males, the average value of Q was 0·57 mm. and the range 0 to 6·0 mm.; in females, the average was 0·56 mm. and the range 0 to 3·8 mm.

3*rd Decade.*—In males, the average was 0·56 mm. and the range 0 to 3·0 mm.; in females, the averge was 0·54 mm. and the range 0 to 2·7 mm.

4th Decade.—In males the average was 0·42 mm. and the range 0 to 4·2 mm.; for females, the average was 0·48 mm. and the range 0 to 3·0 mm.

5th Decade.—For males, the average was 0·31 mm. and the range 0 to 3·4 mm.; for females, the average was 0·39 mm. and the range 0 to 5·0 mm.

The size of the Q wave was, on an average, slightly greater in females than in males. There was no increase in size with age. In the series of cases reported by Chamberlain and Hay, the Q wave was larger in the 5th, 6th and 7th decades than in the 2nd, 3rd and 4th. In my series, the value of Q in the 5th decade was actually lower than in the earlier decades of life.

The value of Q_3 was under 4·2 mm. in all records except two (one male with 6·0 mm. and one female with 5·0 mm.). In Chamberlain's and Hay's series the value of Q_3 was never above 4·5 mm.

The R Deflection or Wave

Amplitude of R.—This is measured from the iso-electric line to the summit of the R wave. In my series of 200 records, the average amplitude of R taking all leads into consideration, was 9·2 mm. The average amplitudes of R in the individual leads were as follows: Lead I = 7·4 mm.; Lead II = 12·0 mm.; Lead III = 8·1 mm.

Range.—In Lead I, the amplitude of R ranged from 1·6 to 20·2 mm., in Lead II from 2·9 to 23·2 mm. and in Lead III 0·8 to 19·7 mm. (See Table XV.)

2nd Decade.—For males, the average was 8·3 mm. and the range 0·8 to 23·2 mm.; for females, the average was 7·2 mm. and the range 1·0 to 16·2 mm.

3rd Decade.—For males, the average was 9·2 mm. and the range 1·5 to 22·6 mm.; for females, the average was 9·5 mm. and the range 3·6 to 21·1 mm.

4th Decade.—For males, the average was 10·8 mm. and the range 3·0 to 17·6 mm.; for females, the average was 10·6 mm. and the range 3·2 to 18·2 mm.

5th Decade.—For males, the average value was 8·7 mm. and the range 2·2 to 22·2 mm.; for females, the average was 8·7 mm. and the range 2·9 to 19·6 mm.

Average values for R wave amplitude were lower in females in Leads I and II and lower in males in Lead III.

(For further particulars, see Table XVI.)

Out of 200 records in my series, there were 28 records (14%) with the R waves in all leads less than 7 mm. in amplitude. The maximum deflection of R in these cases measured from 5·0 to 6·9 mm. There was not a single case with the maximum amplitude of R less than 5 mm.

The S Wave or Deflection

Incidence of the S Wave.—An S wave was observed in the great majority of my records. It was present in Lead I in 70% of records, in Lead II in 77% and in Lead III in 71%.

In 13 records (6·5%), there was a complete absence of the S deflection. (See Table XVII.) There was a higher incidence of the S wave in males than in females and in the later decades of life.

Amplitude or Size of S Wave.—The average amplitude of S for the whole series, taking all leads into consideration, was 1·97 mm. The average amplitudes of S for the individual leads were: Lead I = 1·63 mm., Lead II = 2·51 mm.; Lead III = 1·77 mm.

Range.—In Lead I, the amplitude of S ranged from 0 to 8·6 mm.; in Lead II from 0 to 10·1 mm. and in Lead III from 0 to 8·6 mm. (See Table XVIII.)

2*nd Decade.*—For males, the average was 1·55 mm. and the range 0 to 10·1 mm.; for females, the average was 1·11 mm., and the range 0 to 7·1 mm.

3*rd Decade.*—For males, the average was 2·15 mm., and the range 0 to 9·2 mm.; for females, the average was 2·22 mm., and the range 0 to 9·3 mm.

4*th Decade.*—For males, the average was 2·09 mm., and the range 0 to 7·8 mm.; for females, the average was 2·5 mm., and the range 0 to 8·2 mm.

5*th Decade.*—For males, the average was 2·00 mm., and the range 0 to 5·6 mm.; for females, the average was 2·05 mm., and the range 0 to 7·2 mm.

Notching and Slurrring of QRS

Notching of QRS.—In my series of 200 records, notching of QRS was observed in Lead I on three occasions only and in Lead II but once. In Lead III notching of QRS was noted 32 times (16%). In the great majority of cases, notched QRS complexes were either " vibratory " of form or of small amplitude; in two cases notching of QRS was observed with amplitudes of 6·2 and 7·5 mm. (See Table XIX.)

Basal Slurring of QRS.—By this term is meant a thickening or splaying out of one or both limbs of the R or S wave, close to or continuous with the iso-electric line. It may occur in perfectly normal records.

In my series, " basal slurring " of R was observed in as many as 62 records (31%). In 3 records (1·5%) basal slurring of R was common to all the three standard leads. In 21 records (10·5%), it was observed in two leads, 6 times in Leads I and II, 8 times in Leads II and III and 7 times in Leads I and III. In 38 records (19%), basal slurring was confined to one lead only, 12 times to Lead I, 17 times to Lead II and 9 times to Lead III.

Apical Slurring.—By this term is meant a thickening or splaying out of one or more limbs of the R or S wave, not continuous with the base-line.

In the present series, apical slurring of R was observed in as many as 22 records (11%); in all these cases, the slurring was confined to one lead only; it occurred on 4 occasions in Lead I, twice in Lead II and 16 times in Lead III.

2nd Decade.—Apical slurring was observed in 12% of cases in males (once in Lead I and twice in Lead III), and 16% of cases in females once in Lead I and three times in Lead III.

3rd Decade.—Apical slurring occurred in 8% of male records (twice in Lead III) and 8% of female records (twice in Lead III).

4th Decade.—It was noted in 12% of male records (once in Lead I, once in Lead II and once in Lead III); in female records, it occurred in 8% (once in Lead I and once in Lead III).

5th Decade.—It was noted in 12% male records (3 times in Lead I) and in 12% of female records (once in Lead II and twice in Lead III).

R waves with apical slurring were of small amplitude in the great majority of cases (from 1·1 to 5·0 mm.); in 3 records, the amplitude of R was more than 5 mm. (*viz.*, 7·2, 6·2 and 5·8 mm., respectively).

Out of 22 records with apical slurring of R, slurring was observed 8 times in the upstroke and 14 times in the downstroke. Apical slurring of the down-stroke was almost twice as common as that of the upstroke, in the present series of records.

The S–T Interval

This important interval is measured from the end of S to the beginning of the T wave.

Duration of the S–T Interval.—The measurement of the S–T interval may be extremely difficult or impossible in records, where the T wave appears to arise directly from the preceding QRS complex. Such an apparent absence

of the S–T interval was observed in 25 records of my series (12·5%). The duration of S–T could not be measured in Lead I 7 times, in Lead II 6 times and in Lead III 18 times.

In my series, the average value for the S–T interval, taking all leads into consideration, was 0·088 sec. The average values for the individual leads were as follows: Lead I = 0·087 sec.; Lead II = 0·087 sec.; Lead III = 0·091 sec. High values were most frequently encountered in Lead III.

Range.—In Lead I, the S–T interval ranged from 0·02 to 0·16 sec., in Lead II from 0·02 to 0·16 sec., and in Lead III from 0·02 to 0·16 sec. (See Table XX.)

2nd Decade.—The average value of S–T for males was 0·085 sec., with a range of 0·02 to 0·16 sec.; for females, the average was 0·089 sec., and the range 0·02 to 0·16 sec.

3rd Decade.— For males, the average was 0·089 sec., and the range 0·04 to 0·16 sec. ; for females, the average was 0·090 sec., and the range 0·04 to 0·16 sec.

4th Decade.—For males, the average was 0·085 sec. and the range 0·03 to 0·16 sec.; for females, the average was 0·088 sec., and the range 0·04 to 0·16 sec.

5th Decade.—For males, the average was 0·088 sec., and the range 0·04 to 0·16 sec.; for females, the average was 0·089 sec., and the range 0·02 to 0·16 sec.

In 4 records (2%), the S–T interval was identical in all leads (values being 0·04, 0·08, 0·09 and 0·09 sec. respectively.) In 32 records, maximum values for S–T were observed in Lead I; of these, 30 showed similar values for S–T in Lead II and 4 in Lead III. In 46% of records, maximum values were noted in Lead II; of these 30 showed similar values in Lead I and 28 in Lead III. In 58% of records, maximum values for S–T were noted in Lead III; of these, 26 showed similar values in Lead II and 4 in Lead I.

Relation of S–T Segment to the Base-line

Deviation of the S–T segment from the base-line (or P–R level) was measured in each of the two hundred records of my series. In Lead I, the S–T segment was at the level of P–R in 46%, raised in 18% and depressed in 36% of records. Depression of S–T was more frequently observed than elevation of S–T (ratio of 2 : 1).

In Lead II, the S–T segment was at the level of P–R in 34%, raised in 20% and depressed in 46%. Depression of S–T was much more common than elevation (ratio of 2·3 : 1·0).

In Lead III, the S–T segment was at the level of P–R in 44%, raised in 30% and depressed in 26% of reocrds. The above findings are diametrically opposed to those of Hoskin and Jonescu, who found elevation of S–T more common than depression of S–T in Leads I and II and depression of S–T commoner than elevation in Lead III.

Degree of S-T Deviation.—S–T deviation seldom exceeds 1 mm. In the present series of records, upward deviation of S–T ranged from 0·1 to 1·2 mm. The maximum figure of 1·2 mm. was attained only once in Lead I. Upward deviation of over 1 mm. was encountered 10 times (5%). No age or sex differences were apparent.

Depression of S–T segment ranged from 0·1 to 1·6 mm. in the 200 records. The maximum value of 1·6 mm. was attained 4 times, 3 times in Lead II and once in Lead III. Values over 1 mm. were noted 12 times (6%).

Elevation of S–T in all three leads was observed in only 3 records (1·5%), while depression in all leads was noted 10 times (5%). In 18 records (9%), there was elevation of S–T in one lead, depression of S–T in one lead and an iso-electric S–T in the remaining lead. In 23 records (11·5%), S–T was raised in two leads and depressed in the third lead. In 28 records (14%), S–T was depressed in two leads and raised in the remaining lead. In 8 records (4%), S–T was raised in two leads and iso-electric in one lead. In 30 records (15%), S–T was depressed in two leads and iso-electric in one lead. In 54 records (27%), S–T deviation was confined to one lead only; of these, 18 showed elevation and 36 showed depression. In 26 records (13%), S–T was iso-electric in all leads.

The S-T Duration

This is measured from the end of S to the end of the T wave.

The average value for S–T duration, in the present series, was 0·26 sec., taking all leads into consideration. The average value for Lead I was 0·26 sec., for Lead II 0·26 sec., and for Lead III 0·27 sec.

Range.—In Lead I, the S–T duration ranged from 0·16 sec. to 0·36 sec., in Lead II from 0·15 to 0·36 sec., and in Lead III from 0·156 to 0·36 sec. (See Table XXI.)

2nd Decade.—The average value of the S–T duration for males was 0·25 sec., with a range of 0·15 to 0·36 sec.; for females, the average was 0·25 sec. and the range 0·18 to 0·36 sec.

3rd Decade.—For males, the average was 0·26 sec., and the range 0·17 to 0·36 sec.; for females, the average was 0·26 sec., and the range 0·16 to 0·36 sec.

4th Decade.—For males, the average was 0·27 sec., and the range 0·20 to 0·36 sec.; for females, the average was 0·26 sec., and the range 0·19 to 0·36 sec.

5th Decade.—For males, the average was 0·28 sec., and the range 0·22 to 0·34 sec.; for females, the average was 0·27 sec., and the range 0·20 to 0·36 sec.

In the series of normals reported by Chamberlain and Hay, the value of S–T duration was fairly constant (about 0·28 sec.) and showed no alteration with age. In the present series, there was a slight increase in the duration of S–T with age (a difference of about 0·03 sec., in S–T duration between the values of the 2nd and 5th decades). While the average value for S–T was 0·28 in the series reported by Chamberlain and Hay, it was 0·26 sec. in the present series.

In the series of normals reported by Hoskin and Jonescu the average value of S–T was maximum in Lead I and minimum in Lead III. In my series, the maximum average value of S–T duration was observed in Lead III.

The T Wave or the Second Ventricular Deflection

Direction of the T Wave.—In my series of 200 normal records, the T wave was invariably upright in Lead I with but one exception, where the T wave was iso-electric or flat. In Lead II, the T wave was upright in all but five cases; in two of these, it was iso-electric, in two biphasic and in one inverted. Hence it is justifiable to assume that in Leads I and II, the T wave is practically invariably positive or upright. The reverse holds true in case of Lead III, where there is no constancy at all about the duration or form of the T wave. For an analysis of the T wave duration in Lead III see Table XXII.

"Pointed" and "Rounded" Forms of T Wave.—Two main forms of T wave can be distinguished on the basis of contour, *viz.*, the "pointed form" and the "rounded form" of T.

In my series of 200 records, the T wave used in Lead I was "pointed" in 70%, "rounded" in 24% and of the "mixed form" or "pointed and rounded" in 6%. In Lead II T was "pointed" in 49%, "rounded" in 50% and "flat" in 1%. In Lead III, it was "pointed" on 37%, "rounded" in 46%, "flat" in 7·5% and "biphasic" in 9·5%.

Ratio of "pointed" to "rounded" forms in the three leads was as follows: Lead I = 3 : 1; Lead II = 1 : 1; Lead III = 4 : 5.

Amplitude of the T Wave.—The average value of T for my series, taking all leads into consideration, was 2·09 mm. The average value of T for Lead I was 2·8 mm., for Lead II 3·5 mm., and for Lead III – 0·04 mm.

Range.—The amplitude of T wave ranged from 0 to 9·1 mm. in Lead I, from − 0·4 to 10·1 mm. in Lead II, and from − 4·2 to 6·2 mm. in Lead III. (See Table XXIII.)

2*nd Decade.*—For males, the average value of T was 2·31 mm. and the range − 4·2 mm. (observed once in Lead III) to 9·8 mm. For females, the average was 2·10 mm. and the range from − 2·6 mm. (in Lead III) to 7·3 mm.

3*rd Decade.*—For males, the average was 2·4 mm. the range − 2·2 mm. (in Lead III) to 8·1 mm.; for females, the average was 2·3 mm. and the range − 3·1 mm. (in Lead III) to 10·1 mm.

4*th Decade.*—For males, the average was 1·7 mm. and the range − 3·2 mm. (in Lead III) to 7·2 mm.; for females, the average was 1·5 mm. and the range − 4·1 mm. (in Lead III) to 6·1 mm.

5*th Decade.*—For males, the average was 1·8 mm. and the range − 4·0 mm. (in Lead III) to 7·1 mm.; for females the average was 2·2 mm. and the range − 2·6 mm. (in Lead III) to 10·1 mm.

The average values for T wave amplitude were higher in the 2nd and 3rd than in the fourth and 5th decades of life. Average values for male and female subjects were practically identical. Maximum values for T wave amplitude were observed in Lead II and minimum values in Lead III.

(For further particulars, see Table XXIV.)

Amplitude of T in the Lead of Largest Measurement.—In this series, the size of T in the lead of largest measurement varied from a minimum of 1·7 mm. to a maximum of 10·1 mm. The maximum figure of 10·1 mm. was observed in Lead II on two occasions only. In Chamberlain's and Hay's series, the maximum figure obtained in Lead II was 12·5 mm.

Values under 2·0 mm. were observed in 6 records only (3%), the figures being 1·8, 1·7, 1·8, 1·9, 1·8 and 1·8 mm. respectively. Values over 5·0 mm. were noted in as many as 21% of the cases. Maximum values for T were noted in Lead I in 43% of records, in Lead II in 56·5% and in Lead III in only 0·5%. Maximum values for T are practically never encountered in Lead III.

The average size of T in the lead of largest excursion, in the present series, was 3·6 mm.

Duration of T Wave.—The average duration of T for my series, taking all leads into consideration, was 0·17 sec. Average values for the individual leads were: Lead I = 0·172 sec.; Lead II = 0·173 sec.; Lead III = 0·163 sec.

Range.—In Lead I, the duration of T ranged from 0·10 to 0·26 sec.; in Lead II from 0·10 to 0·28 sec.; in Lead III from 0·10 to 0·26 sec. (See Table XXV.)

2nd Decade.—For males, the average duration of T was 0·173 sec., with a range of 0·10 to 0·26 sec.; for females, the average was 0·177 sec. and the range 0·10 to 0·28 sec.

3rd Decade.—For males, the average was 0·173 sec., and the range 0·12 to 0·26 sec.; for females, the average was 0·166 sec., and the range 0·12 to 0·22 sec.

4th Decade.—For males, the average was 0·159 sec., and the range 0·10 to 0·24 sec.; for females, the average was 0·167 sec., and the range 0·12 to 0·24 sec.

5th Decade.—For males, the average was 0·167 sec., and the range 0·10 to 0·26 sec.; for females, the average was 0·171 sec., and the range 0·14 to 0·24 sec.

The U Wave

This is also known as "the sixth wave of the electrocardiogram". It was first noted in 1906 by Einthoven. Its cause is not clear. It is observed in the majority of normal records. Lewis and Gilder noted the presence in about 90% of normal records from adults.

Incidence of U Wave.—U waves were observed in the majority of my cases. In 17 records (8·5%), U waves were observed in all the three leads. In 49 records (24·5%), U waves were observed in two leads, 22 times in Leads I and II, 20 times in Leads II and III and 7 times in Leads I and III. In 75 records (37·5%), U waves were confined to one lead only, 29 times to Lead I, 27 times to Lead II and 19 times to Lead III.

The highest incidence of U waves was observed in the 4th decade of life and the lowest in the 2nd decade.

The incidence rate of U waves was practically identical in males and females. (See Table XXVI.)

Form of U Wave.—Two main forms of U wave were encountered: (1) the "Pointed" form with short duration and (2) the "rounded" form with long duration.

In 73 records (36·5%), U waves were of the "rounded" variety only; in 49 records (24·5%), they were all of the "pointed" variety; in 19 records (9·5%), both pointed and rounded forms were encountered. The ratio of "rounded" to "pointed" of U, in my series, was 2·5 : 1·0.

Amplitude of the U Waves.—The average amplitude of U for my series was 0·56 mm. The average value for the "pointed" form of U was 0·81 mm. and for the " rounded " form 0·34 mm.

Range.—The amplitude of U ranged from 1·1 to 0·5 mm. in the 200 records. The "pointed" form of U varied in size from 0·2 to 1·5 mm. and the " rounded " form from 0·1 to 1·2 mm. (For further particulars, see Table XXVII.)

Duration of the U Wave.—The average duration of U for the whole series was 0·165 sec. The average value for the " pointed " form of U was 0·135 sec., and for the " rounded " form 0·180 sec.

Range.—The duration of U ranged from 0·06 to 0·26 in the 200 records. The " pointed " form of U ranged in size from 0·06 to 0·22 sec., and the " rounded " form from 0·08 to 0·26 sec. (For further particulars, see Table XXVIII.)

Summary

1. A study is presented of two hundred normal electrocardiograms of subjects from the second, third, fourth and fifth decades of life. Each decade of life is represented by twenty-five male and twenty-five female subjects.

2. The various deflections and " intervals " of the normal electrocardiogram are measured, described and analysed. Variations due to age and sex are studied.

3. The results of the investigation are briefly presented in tabular form. (See Tables XXIX and XXX.)

It is with very great pleasure that I acknowledge my indebtedness to the Trustees of the late Sir Ratan Tata for a medical research grant, which has made possible the present investigation. I am also grateful to my patients, friends and medical students for thier kind co-operation and patience in the collection of the electrocardiographic material for this study.

[For References, please refer to " An Analysis of Normal Electrocardiograms," *Proc. Ind. Acad. Sci.*, 1940, **12**, 267.]

TABLE I

Showing Distribution of Subjects in the Present Series

Decade of Life	Males	Females	Total
Second	25	25	50
Third	25	25	50
Fourth	25	25	50
Fifth	25	25	50
Series in general	100	100	200

TABLE II

A Detailed Analysis of the Different Forms of the P Wave (Case Numbers)

Decade	Sex	Lead	Pointed Form	Rounded Form	Pointed and Rounded Form	Flat or iso-electric Form	Bifid Form	Inverted Form	Diphasic Form	Number of Case Records	Total number of Records
2nd	M	I	10	9	2	2	1	1	0	25	50
		II	14	9	1	0	1	0	0		
		III	9	6	2	3	1	3	1		
	F	I	14	7	2	0	1	0	1	25	
		II	22	1	0	1	1	0	0		
		III	10	2	1	3	2	3	4		
3rd	M	I	12	10	1	1	0	0	1	25	50
		II	15	9	1	0	0	0	0		
		III	8	8	0	2	1	3	3		
	F	I	12	10	2	0	0	0	1	25	
		II	16	6	1	1	1	0	0		
		III	11	4	2	1	0	2	5		
4th	M	I	12	11	1	1	0	0	0	25	50
		II	10	14	1	0	0	0	0		
		III	8	10	3	1	0	2	1		
	F	I	12	11	2	0	0	0	0	25	
		II	10	10	2	1	1	0	1		
		III	9	10	1	2	0	2	1		
5th	M	I	12	10	2	1	0	0	0	25	50
		II	8	13	2	1	0	1	0		
		III	9	11	1	1	0	1	2		
	F	I	12	11	1	0	1	0	0	25	
		II	10	11	1	0	1	1	1		
		III	8	7	1	0	0	7	2		
2nd to 5th	M	I	46	40	6	5	1	1	1	100	200
		II	47	45	5	1	1	1	0		
		III	34	35	6	7	2	9	7		
	F	I	50	39	7	0	2	0	2	100	
		II	58	28	4	3	4	1	2		
		III	38	23	5	6	2	14	12		

TABLE III

An Analysis of the Amplitude of the P Wave (Values in mms.)

Decade	Value	Males			Females		
		Lead I	Lead II	Lead III	Lead I	Lead II	Lead III
2nd	Aver.	0·85	1·12	0·72	0·80	1·26	0·62
	Min.	−0·3	0·0	−1·1	0·0	0·0	−1·0
	Max.	2·1	3·0	3·0	1·8	2·2	3·2
3rd	Aver.	0·91	1·51	0·84	0·84	1·22	0·55
	Min.	0·0	−0·2	−2·0	0·0	0·0	−1·3
	Max.	2·0	3·1	2·6	1·6	2·3	3·0
4th	Aver.	0·86	1·92	0·98	0·79	1·10	1·00
	Min.	0·0	0·0	−0·5	0·0	0·0	−0·2
	Max.	2·3	3·1	2·0	1·8	2·0	2·1
5th	Aver.	1·00	1·20	0·96	0·90	1·42	0·82
	Min.	0·0	0·0	−1·2	0·0	−0·2	−1·0
	Max.	2·1	2·3	2·2	1·6	2·2	1·8
For all decades	Aver.	0·95	1·44	0·77	0·83	1·25	0·74
	Min.	−0·3	−0·2	−2·0	0·0	−0·2	−1·3
	Max.	2·3	3·1	3·0	1·8	2·3	3·2

TABLE IV

Amplitude of the P Wave (Percentage Frequencies)

Amplitude Range (in mms.)	Lead I	Lead II	Lead III
−2 to −1	0·0	0·0	2·5
−1 to 0	0·5	1·0	10·0
0 to 1	61·0	40·5	53·5
1 to 2	35·5	43·5	28·5
2 to 3	3·0	13·0	4·0
3 to 4	0·0	2·0	1·5

TABLE V

An Analysis of the P Wave Duration (Values in seconds)

Decade	Value	Males			Females		
		Lead I	Lead II	Lead III	Lead I	Lead II	Lead III
2nd	Aver.	0·078	0·092	0·071	0·079	0·090	0·071
	Min.	0·04	0·04	0·04	0·04	0·05	0·03
	Max.	0·15	0·14	0·13	0·14	0·14	0·12
3rd	Aver.	0·083	0·098	0·074	0·080	0·094	0·073
	Min.	0·06	0·05	0·03	0·04	0·06	0·04
	Max.	0·15	0·15	0·14	0·14	0·11	0·10
4th	Aver.	0·079	0·099	0·084	0·078	0·090	0·081
	Min.	0·04	0·04	0·04	0·04	0·03	0·06
	Max.	0·12	0·14	0·14	0·14	0·15	0·12
5th	Aver.	0·080	0·088	0·082	0·078	0·084	0·079
	Min.	0·04	0·04	0·06	0·04	0·06	0·04
	Max.	0·14	0·15	0·14	0·14	0·13	0·14
For all decades	Aver.	0·080	0·094	0·078	0·079	0·090	0·076
	Min.	0·04	0·04	0·03	0·04	0·03	0·03
	Max.	0·15	0·15	0·14	0·14	0·15	0·14

TABLE VI

An Analysis of the P–Q Duration (Values in seconds)

Decade	Value	Males			Females		
		Lead I	Lead II	Lead III	Lead I	Lead II	Lead III
2nd	Aver.	0·053	0·060	0·071	0·043	0·044	0·053
	Min.	0·01	0·02	0·02	0·01	0·00	0·01
	Max.	0·02	0·12	0·12	0·09	0·10	0·10
3rd	Aver.	0·050	0·060	0·068	0·048	0·050	0·051
	Min.	9·02	0·04	0·02	0·02	0·02	0·03
	Max.	0·12	0·12	0·12	0·11	0·12	0·11
4th	Aver.	0·060	0·060	0·066	0·056	0·060	0·060
	Min.	0·04	0·04	0·01	0·03	0·01	0·02
	Max.	0·10	0·12	0·13	0·10	0·10	0·10
5th	Aver.	0·054	0·058	0·062	0·050	0·052	0·054
	Min.	0·02	0·01	0·04	0·01	0·03	0·02
	Max.	0·12	0·11	0·10	0·12	0·12	0·12
For all decades	Aver.	0·054	0·060	0·067	0·049	0·052	0·055
	Min.	0·01	0·01	0·01	0·01	0·00	0·01
	Max.	0·12	0·12	0·13	0·12	0·12	0·12

TABLE VII

An Analysis of the P–R Interval (Values in seconds)

Decade	Value	Males			Females		
		Lead I	Lead II	Lead III	Lead I	Lead II	Lead III
2nd	Aver.	0·138	0·146	0·144	0·120	0·124	0·124
	Min.	0·08	0·07	0·07	0·08	0·08	0·08
	Max.	0·21	0·21	0·21	0·19	0·20	0·17
3rd	Aver.	0·141	0·148	0·152	0·134	0·140	0·140
	Min.	0·11	0·10	0·12	0·10	0·09	0·09
	Max.	0·20	0·20	0·19	0·20	0·21	0·20
4th	Aver.	0·152	0·164	0·180	0·146	0·158	0·160
	Min.	0·12	0·11	0·12	0·11	0·10	0·09
	Max.	0·20	0·21	0·20	0·20	0·20	0·19
5th	Aver.	0·150	0·152	0·168	0·148	0·154	0·160
	Min.	0·12	0·11	0·10	0·10	0·11	0·09
	Max.	0·20	0·19	0·21	0·19	0·20	0·21
For all decades	Aver.	0·145	0·153	0·161	0·137	0·144	0·146
	Min.	0·08	0·07	0·07	0·08	0·08	0·08
	Max.	0·21	0·21	0·21	0·20	0·21	0·21

TABLE VIII

Maximum P–R Interval Values in 200 Records (Case Numbers)

Range of Duration (in seconds)	Lead I	Lead II	Lead III
0·08 to 0·10	3	7	5
0·11 to 0·13	8	14	16
0·14 to 0·16	30	47	28
0·17 to 0·19	24	34	32
0·20 to 0·22	7	18	19

TABLE IX

Incidence Rate of the P–R Deflection (Case Records)

P–R Deflection	2nd Decade		3rd Decade		4th Decade		5th Decade		For all Decades	
	M	F	M	F	M	F	M	F	M	F
1. In all leads ..	6	8	10	8	11	10	12	12	39	38
2. In two leads ..	12	11	10	11	8	9	9	6	39	37
3. In one lead ..	3	3	2	4	2	0	0	3	7	10
4. In no lead ..	4	3	3	2	4	6	4	4	15	15

TABLE X

An Analysis of the P–R Level (Values in mms.)

Decade	Value	MALES			FEMALES		
		Lead I	Lead II	Lead III	Lead I	Lead II	Lead III
2nd	Aver.	0·22	0·36	0·26	0·22	0·32	0·28
	Min.	0·0	0·0	0·0	0·0	0·0	0·0
	Max.	1·4	2·0	1·0	0·1	1·4	2·1
3rd	Aver.	0·24	0·35	0·28	0·26	0·34	0·30
	Min.	0·0	0·0	0·0	0·0	0·0	0·0
	Max.	1·6	2·0	1·2	1·8	1·8	1·6
4th	Aver.	0·34	0·38	0·30	0·30	0·38	0·32
	Min.	0·0	0·0	0·0	0·0	0·0	0·0
	Max.	1·8	2·0	1·8	2·0	2·0	1·8
5th	Aver.	0·34	0·38	0·32	0·32	0·32	0·34
	Min.	0·0	0·0	0·0	0·0	0·0	0·0
	Max.	2·0	2·0	2·0	2·0	2·2	2·0
For all decades	Aver.	0·29	0·37	0·29	0·27	0·34	0·31
	Min.	0·0	0·0	0·0	0·0	0·0	0·0
	Max.	2·0	2·0	2·0	2·0	2·2	2·0

TABLE XI

An Analysis of the QRS Duration (Values in seconds)

Decade	Value	MALES			FEMALES		
		Lead I	Lead II	Lead III	Lead I	Lead II	Lead III
2nd	Aver.	0·066	0·069	0·068	0·066	0·067	0·067
	Min.	0·03	0·04	0·03	0·04	0·03	0·04
	Max.	0·10	0·11	0·11	0·09	0·10	0·11
3rd	Aver.	0·070	0·070	0·069	0·068	0·068	0·068
	Min.	0·06	0·05	0·05	0·04	0·04	0·05
	Max.	0·10	0·11	0·10	0·10	0·09	0·09
4th	Aver.	0·076	0·074	0·078	0·078	0·072	0·080
	Min.	0·04	0·05	0·06	0·06	0·06	0·06
	Max.	0·10	0·11	0·11	0·10	0·10	0·10
5th	Aver.	0·079	0·080	0·078	0·076	0·076	0·074
	Min.	0·04	0·05	0·06	0·05	0·06	0·05
	Max.	0·10	0·11	0·11	0·10	0·10	0·09
For all decades	Aver.	0·073	0·073	0·073	0·072	0·071	0·072
	Min.	0·03	0·04	0·03	0·04	0·03	0·04
	Max.	0·10	0·11	0·11	0·10	0·10	0·11

TABLE XII

Duration of QRS in the Lead of Largest Measurement (Case Numbers)

Range of Q R S Duration (in seconds)	Lead I	Lead II	Lead III
0·04 to 0·06	22	32	28
0·07 to 0·08	115	80	92
0·09 to 0·10	66	63	76
0·11 to 0·12	0	5	4

TABLE XIII

Table showing Incidence Rate of the Q Wave in Separate Age and Sex Groups (Percentages)

Decade	Males			Females		
	Lead I	Lead II	Lead III	Lead I	Lead II	Lead III
Second	60	64	80	52	64	68
Third	56	60	72	40	48	56
Fourth	40	52	60	48	60	60
Fifth	56	56	68	56	60	64

TABLE XIV

An Analysis of the Q Wave Amplitude (Values in mms.)

Decade	Value	Males			Females		
		Lead I	Lead II	Lead III	Lead I	Lead II	Lead III
2nd	Aver.	0·45	0·39	0·86	0·46	0·38	0·84
	Min.	0·0	0·0	0·0	0·0	0·0	0·0
	Max.	4·1	2·6	6·0	3·4	2·3	3·8
3rd	Aver.	0·44	0·60	0·64	0·42	0·62	0·58
	Min.	0·00	0·0	0·0	0·0	0·0	0·0
	Max.	3·0	2·6	3·0	2·0	2·4	2·7
4th	Aver.	0·26	0·40	0·60	0·32	0·40	0·72
	Min.	0·0	0·0	0·0	0·0	0·0	0·1
	Max.	3·1	3·0	4·2	2·0	2·2	3·0
5th	Aver.	0·14	0·42	0·36	0·26	0·52	0·40
	Min.	0·0	0·0	0·0	0·0	0·0	0·0
	Max.	2·2	2·4	3·4	1·8	2·6	5·0
For all decades	Aver.	0·32	0·45	0·62	0·37	0·48	0·64
	Min.	0·0	0·0	0·0	0·0	0·0	0·0
	Max.	4·1	3·0	6·0	3·4	2·4	5·0

TABLE XV

An Analysis of the R Wave Amplitude (Values in mms.)

Decade	Value	Males			Females		
		Lead I	Lead II	Lead III	Lead I	Lead II	Lead III
2nd	Aver.	7·6	11·6	6·2	6·4	9·8	5·3
	Min.	1·6	2·9	0·8	1·8	3·4	1·0
	Max.	20·2	23·2	18·4	13·6	16·2	12·6
3rd	Aver.	8·4	12·1	7·2	7·8	11·5	9·2
	Min.	3·2	5·1	1·5	3·6	5·3	3·6
	Max.	17·8	22·6	19·7	15·2	21·1	18·9
4th	Aver.	7·2	13·8	11·4	6·6	13·2	12·1
	Min.	3·0	6·2	5·0	3·6	4·8	3·2
	Max.	12·2	17·6	16·8	13·0	18·2	15·8
5th	Aver.	8·3	11·8	6·1	6·8	12·1	7·2
	Min.	5·2	4·9	2·2	3·6	3·8	2·9
	Max.	19·6	22·2	10·8	14·2	19·6	13·0
For all decades	Aver.	7·9	12·3	7·7	6·9	11·7	8·5
	Min.	1·6	2·9	0·8	1·8	3·4	1·0
	Max.	20·2	23·2	19·7	15·2	21·1	18·9

TABLE XVI

An Aanlysis of the R Wave Amplitude (Percentage)

Size of R Wave (Range in mm.)	Lead I	Lead II	Lead III
0·0 to 5·0	929·0	11·5	24·0
5·0 to 10·0	3·5	20·5	38·5
10·0 to 15·0	19·0	38·5	23·0
15·0 to 20·0	8·5	18·5	14·5
20·0 to 25·0	4·0	11·0	0·0

TABLE XVII

Table showing Incidence Rate of the S Wave in Differnt Age and Sex Groups (Percentages)

Decade	Males			Females			Males and Fefmles		
	Lead I	Lead II	Lead III	Lead I	Lead II	Lead III	Lead I	Lead II	Lead III
2nd	76	82	64	62	60	48	69	71	56
3rd	70	76	78	68	76	72	69	76	75
4th	76	82	76	70	78	72	73	80	74
5th	70	75	76	68	82	79	69	79	78

TABLE XVIII

An Analysis of the S Wave Amplitude (Values in mm.)

Decade	Value	Males			Females		
		Lead I	Lead II	Lead III	Lead I	Lead II	Lead III
2nd	Aver.	1·62	1·84	1·20	1·12	1·30	0·92
	Min.	0·0	0·0	0·0	0·0	0·0	0·0
	Max.	6·1	10·1	8·6	7·1	3·6	3·5
3rd	Aver.	1·90	2·92	1·62	1·78	2·85	2·02
	Min.	0·0	0·0	0·0	0·0	0·0	0·0
	Max.	8·6	9·2	8·5	7·8	9·3	6·6
4th	Aver.	2·10	2·96	1·20	1·80	3·10	2·60
	Min.	0·0	0·0	0·0	0·0	0·0	0·0
	Max.	5·6	7·8	3·4	4·6	8·2	6·2
5th	Aver.	1·20	2·60	2·20	1·32	2·50	2·32
	Min.	0·0	0·0	0·0	0·0	0·0	0·0
	Max.	4·1	5·6	5·2	6·0	7·2	6·3
For all decades	Aver.	1·74	2·58	1·56	1·51	2·44	1·97
	Min.	0·0	0·0	0·0	0·0	0·0	0·0
	Max.	8·6	10·1	8·6	7·8	9·3	6·6

TABLE XIX

Table showing Incidence Rate of the QRS Notching in Separate Age and Sex Groups (Case Records)

Decade	Males			Females			Males and Females		
	Lead I	Lead II	Lead III	Lead I	Lead II	Lead III	Lead I	Lead II	Lead III
2nd	0	0	4	0	0	4	0	0	8
3rd	0	0	5	0	0	3	0	0	8
4th	1	0	4	0	0	2	1	0	6
5th	1	0	5	1	1	5	2	1	10

TABLE XX

An Analysis of the S–T Duration (Interval in seconds)

Decade	Value	Males			Females		
		Lead I	Lead II	Lead III	Lead I	Lead II	Lead III
2nd	Aver.	0·082	0·084	0·090	0·085	0·087	0·094
	Min.	0·03	0·04	0·02	0·02	0·02	0·03
	Max.	0·16	0·15	0·16	0·14	0·16	0·16
3rd	Aver.	0·088	0·086	0·092	0·088	0·090	0·092
	Min.	0·06	0·05	0·04	0·06	0·04	0·04
	Max.	0·16	0·14	0·14	0·12	0·12	0·16
4th	Aver.	0·080	0·086	0·090	0·090	0·086	0·088
	Min.	0·04	0·03	0·04	0·06	0·06	0·04
	Max.	0·12	0·14	0·16	0·14	0·14	0·15
5th	Aver.	0·090	0·088	0·086	0·088	0·086	0·092
	Min.	0·04	0·04	0·04	0·06	0·06	0·02
	Max.	0·16	0·14	0·14	0·15	0·14	0·16
For all decades	Aver.	0·085	0·086	0·090	0·088	0·087	0·092
	Min.	0·03	0·03	0·02	0·02	0·02	0·02
	Max.	0·16	0·15	0·16	0·15	0·16	0·16

TABLE XXI

An Analysis of the S–T Duration (Values in seconds)

Decade	Value	Males			Females		
		Lead I	Lead II	Lead III	Lead I	Lead II	Lead III
2nd	Aver.	0·24	0·25	0·25	0·24	0·25	0·25
	Min.	0·16	0·15	0·16	0·18	0·19	0·18
	Max.	0·36	0·36	0·34	0·34	0·34	0·36
3rd	Aver.	0·26	0·25	0·27	0·26	0·26	0·27
	Min.	0·18	0·17	0·18	0·20	0·16	0·19
	Max.	0·32	0·36	0·30	0·36	0·34	0·36
4th	Aver.	0·27	0·26	0·27	0·26	0·25	0·27
	Min.	0·22	0·23	0·20	0·24	0·19	0·20
	Max.	0·34	0·36	0·36	0·36	0·34	0·36
5th	Aver.	0·28	0·28	0·27	0·27	0·28	0·27
	Min.	0·26	0·24	0·22	0·20	0·20	0·20
	Max.	0·34	0·32	0·34	0·34	0·34	0·36
For all decades	Aver.	0·26	0·26	0·27	0·26	0·26	0·27
	Min.	0·16	0·15	0·16	0·18	0·16	0·18
	Max.	0·36	0·36	0·36	0·36	0·34	0·36

TABLE XXII

An Analysis of the T Wave Duration in Lead III (*Percentages*)

Decade	Sex	Upright Form	Inverted Form	Biphasic Form	Iso-electric Form
2nd	M	40	36	16	8
	F	24	56	8	12
3rd	M	44	36	8	12
	F	40	40	12	8
4th	M	52	40	4	4
	F	48	44	4	4
5th	M	52	28	12	8
	F	52	32	12	4
For all decades	M and F	44	39	9·5	7·5

TABLE XXIII

An Analysis of the T Wave Amplitude (*Size in mms.*)

Decade	Value	Males			Females		
		Lead I	Lead II	Lead III	Lead I	Lead II	Lead III
2nd	Aver.	3·3	3·6	0·03	2·7	3·6	0·01
	Min.	0·4	0·6	−4·2	0·0	−0·4	−2·6
	Max.	9·1	9·8	6·0	6·2	7·3	3·3
3rd	Aver.	3·1	4·1	0·0	3·0	3·6	0·2
	Min.	0·9	0·0	−2·2	0·6	0·8	−3·1
	Max.	7·2	8·1	4·2	6·6	10·1	5·2
4th	Aver.	2·3	3·1	−0·2	2·2	2·5	−0·1
	Min.	0·2	0·5	−3·2	1·0	0·0	−4·1
	Max.	6·0	7·2	3·8	4·6	6·1	4·2
5th	Aver.	2·4	3·0	0·1	2·8	4·1	−0·3
	Min.	0·6	0·5	−4·0	0·5	1·0	−2·6
	Max.	5·8	7·1	5·4	7·8	10·1	6·2
For all decades	Aver.	2·8	3·5	−0·02	2·7	3·5	−0·05
	Min.	0·2	0·0	−4·2	0·0	−0·4	−4·1
	Max.	9·1	9·8	6·0	7·8	10·1	6·2

TABLE XXIV

Amplitude of the T Wave in 200 Cases (Case Numbers) (Values in mms.)

Size of T Wave (Range in mm.)	Lead I	Lead II	Lead III
−6·0 to −4·0	0	0	4
−4·0 to −2·0	0	0	20
−2·0 to 0·0	0	1	61
0·0 to 2·0	48	43	54
2·0 to 4·0	111	82	34
4·0 to 6·0	20	42	22
6·0 to 8·0	15	20	5
8·0 to 10·0	6	10	0
10·0 to 12·0	0	2	0

TABLE XXV

An Analysis of the T Wave Duration (Values in seconds)

Decade	Value	Males			Females		
		Lead I	Lead II	Lead III	Lead I	Lead II	Lead III
2nd	Aver.	0·176	0·174	0·170	0·178	0·180	0·172
	Min.	0·10	0·10	0·10	0·10	0·12	0·10
	Max.	0·25	0·26	0·24	0·25	0·28	0·26
3rd	Aver.	0·182	0·176	0·160	0·160	0·172	0·166
	Min.	0·16	0·12	0·12	0·12	0·16	0·14
	Max.	0·26	0·24	0·24	0·18	0·22	0·20
4th	Aver.	0·168	0·170	0·140	0·170	0·168	0·164
	Min.	0·10	0·12	0·10	0·12	0·14	0·12
	Max.	0·20	0·24	0·24	0·24	0·22	0·20
5th	Aver.	0·170	0·168	0·168	0·172	0·174	0·168
	Min.	0·14	0·14	0·10	0·14	0·16	0·16
	Max.	0·24	0·26	0·25	0·20	0·22	0·24
For all decades	Aver.	0·174	0·172	0·158	0·170	0·174	0·168
	Min.	0·10	0·10	0·10	0·10	0·12	0·10
	Max.	0·26	0·26	0·26	0·25	0·28	0·26

Table XXVI

Table showing Incidence Rate of the U Wave in Differnt Age and Sex Groups (Percentages)

Decade	Males	Females	Both Sexes
Second	56	60	58
Third	76	72	74
Fourth	72	80	76
Fifth	76	72	74
For all decades	70	71	70·5

Table XXVII

An Analysis of the U Wave Amplitude (Size in mms.)

Decade	Value	Males			Females		
		Pointed Form	Rounded Form	U Waves in general	Pointed Form	Rounded Form	U Waves in general
2nd	Aver.	0·92	0·28	0·50	0·64	0·30	0·42
	Min.	0·2	0·1	0·1	0·4	0·2	0·2
	Max.	1·4	1·2	1·4	1·2	0·8	1·2
3rd	Aver.	0·80	0·30	0·55	1·1	0·4	0·75
	Min.	0·5	0·2	0·2	1·0	0·2	0·2
	Max.	1·5	1·0	1·5	1·4	0·8	1·4
4th	Aver.	0·7	0·3	0·50	0·7	0·5	0·(0
	Min.	0·4	0·1	0·1	0·6	0·4	0·4
	Max.	1·2	0·6	1·2	0·8	0·6	0·8
5th	Aver.	0·9	0·3	0·60	0·7	0·3	0·50
	Min.	0·6	0·1	0·1	0·4	0·2	0·2
	Max.	1·2	0·4	1·2	1·1	0·4	1·1
For all decades	Aver.	0·83	0·30	0·54	0·79	0·38	0·57
	Min.	0·2	0·1	0·1	0·4	0·2	0·2
	Max.	1·5	1·2	1·5	1·4	0·8	1·4

TABLE XXVIII

An Analysis of the U Wave Duration (*Values in seconds*)

Decade	Value	Males			Females		
		Pointed Form	Rounded Form	U Waves in general	Pointed Form	Rounded Form	U Waves in general
2nd	Aver.	0·10	0·16	0·14	0·12	0·15	0·14
	Min.	0·06	0·08	0·06	0·06	0·08	0·06
	Max.	0·22	0·22	0·24	0·18	0·20	0·22
3rd	Aver.	0·13	0·17	0·16	0·16	0·19	0·18
	Min.	0·10	0·12	0·10	0·12	0·16	0·12
	Max.	0·20	0·24	0·24	0·22	0·24	0·24
4th	Aver.	0·14	0·18	0·16	0·12	0·18	0·16
	Min.	0·10	0·14	0·10	0·08	0·10	0·08
	Max.	0·20	0·24	0·24	0·18	0·22	0·22
5th	Aver.	0·15	0·21	0·19	0·16	0·20	0·18
	Min.	0·10	0·18	0·10	0·08	0·12	0·08
	Max.	0·20	0·26	0·26	0·20	0·24	0·24
For all decades	Aver.	0·13	0·18	0·16	0·14	0·18	0·17
	Min.	0·06	0·08	0·06	0·06	0·08	0·06
	Max.	0·22	0·26	0·26	0·22	0·24	0·24

TABLE

A Summary of the Main Deflections and

Deflection	Feature Investigated	Sex	2ND DECADE			3RD DECADE		
			Aver.	Min.	Max.	Aver.	Min.	Max.
P Wave ..	Amplitude	M	0·90	−1·1	3·0	0·94	−2·0	3·1
		F	0·89	−1·0	3·2	0·86	−1·3	3·0
	Duration	M	0·080	0·04	0·15	0·085	0·03	0·15
		F	0·080	0·03	0·14	0·082	0·04	0·14
Q R S complex ..	Duration	M	0·068	0·03	0·11	0·070	0·05	0·11
		F	0·067	0·03	0·11	0·068	0·04	0·10
Q Wave ..	Amplitude	M	0·57	0·0	6·0	0·56	0·0	3·0
		F	0·56	0·0	3·8	0·54	0·0	2·7
R Wave ..	Amplitude	M	8·3	0·8	23·2	9·2	1·5	22·6
		F	7·2	1·0	16·2	9·5	3·6	21·1
S Wave ..	Amplitude	M	1·55	0·0	10·1	2·15	0·0	9·2
		F	1·11	0·0	7·1	2·22	0·0	9·3
T Wave ..	Amplitude	M	2·31	−4·2	9·8	2·4	−2·2	8·1
		F	2·10	−2·6	7·3	2·3	−3·1	10·1
	Duration	M	0·173	0·10	0·26	0·173	0·12	0·26
		F	0·177	0·10	0·28	0·166	0·12	0·22
U Wave ..	Amplitude	M	0·50	·10	1·4	0·55	0·20	1·5
		F	0·42	0·20	1·2	0·75	0·20	1·4
	Duration	M	0·14	0·06	0·24	0·16	0·10	0·24
		F	0·14	0·06	0·22	0·18	0·12	0·24
P–Q Interval ..	Duration	M	0·061	0·01	0·12	0·059	0·02	0·12
		F	0·047	0·00	0·10	0·050	0·02	0·12
P–R Interval ..	Duration	M	0·143	0·07	0·21	0·147	0·10	0·20
		F	0·123	0·08	0·20	0·138	0·09	0·21
P–R Level ..	Deviation	M	0·28	0·0	2·0	0·29	0·0	2·0
		F	0·27	0·0	2·1	0·30	0·0	1·8
S–T Interval ..	Duration	M	0·085	0·02	0·16	0·089	0·04	0·16
		F	0·089	0·02	0·16	0·090	0·04	0·16
S–T Duration ..	Duration	M	0·25	0·15	0·36	0·26	0·17	0·36
		F	0·25	0·18	0·36	0·26	0·16	0·36

XXIX

"Intervals" of the Electrocardiogram

4th Decade			5th Decade			For All Decades			Scale
Aver.	Min.	Max.	Aver.	Min.	Max.	Aver.	Min.	Max.	
1·25	−0·5	3·1	1·05	−1·2	2·3	1·05	−2·0	3·1	mm.
0·96	−0·2	2·1	1·05	−1·0	2·2	0·94	−1·3	3·2	,,
0·087	0·04	0·14	0·083	0·04	0·15	0·084	0·03	0·15	sec.
0·083	0·03	0·15	0·080	0·04	0·14	0·082	0·03	0·15	,,
0·076	0·04	0·11	0·079	0·04	0·11	0·073	0·03	0·11	sec.
0·077	0·06	0·10	0·075	0·05	0·10	0·072	0·03	0·11	,,
0·42	0·0	4·2	0·31	0·0	3·4	0·46	0·0	6·0	mm.
0·48	0·0	3·0	0·39	0·0	5·0	0·50	0·0	5·0	,,
10·8	3·0	17·6	8·7	2·2	2·2	9·3	0·8	23·2	mm.
10·6	3·2	18·2	8·7	2·9	19·6	9·0	1·0	21·1	,,
2·09	0·0	7·8	2·00	0·0	5·6	1·96	0·0	10·1	mm.
2·50	0·0	8·2	2·05	0·0	7·2	1·97	0·0	9·3	,,
1·7	−3·2	7·2	1·8	−5·0	7·1	2·1	−4·2	9·8	mm.
1·5	−4·1	6·1	2·2	−2·6	10·1	2·1	−4·1	10·1	,,
0·159	0·10	0·24	0·167	0·10	0·26	0·168	0·10	0·26	sec.
0·167	0·12	0·24	0·171	0·14	0·24	0·171	0·10	0·29	,,
0·50	0·10	1·2	0·60	0·10	1·2	0·54	0·1	1·5	mm.
0·60	0·40	0·80	0·50	0·20	1·1	0·57	0·2	1·4	,,
0·16	0·10	0·24	0·19	0·10	0·26	0·16	0·06	0·26	sec.
0·16	0·08	0·22	0·18	0·08	0·25	0·17	0·06	0·24	,,
0·062	0·01	0·13	0·058	0·01	0·12	6·0	0·01	0·13	sec.
0·059	0·01	0·10	0·052	0·01	0·12	5·2	0·00	0·12	,,
0·165	0·11	0·21	0·157	0·10	0·21	0·153	0·07	0·21	sec.
0·155	0·09	0·20	0·154	0·09	0·21	0·142	0·08	0·21	,,
0·34	0·0	2·0	0·35	0·0	2·0	0·32	0·0	2·0	mm.
0·33	0·0	2·0	0·33	0·0	2·2	0·31	0·0	2·2	,,
0·085	0·03	0·16	0·088	0·04	0·16	0·087	0·02	0·16	sec.
0·088	0·04	0·16	0·089	0·02	0·16	0·089	0·02	0·16	,,
0·27	0·20	0·36	0·28	0·22	0·34	0·26	0·15	0·36	sec.
0·26	0·19	0·36	0·27	0·20	0·36	0·26	0·16	0·36	,,

INFANTILE CIRRHOSIS OF THE LIVER

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Introduction

PHYSICIANS and Pathologists in India have interested themselves in the study of the "Infantile Cirrhosis of the Liver" in view of the appalling mortality of the children claimed by this disease; yet our knowledge of the ætiology and pathology of the disease is singularly defective.

A case report by Sen in 1887 at the Calcutta Medical Association is, so far as I am aware, the first contribution to the literature on the subject, and he has described the disease as "a peculiar enlargement of the liver in young children only, both male and female, without any enlargement of the spleen and associated with low fever, gradual emaciation, loss of appetite, sallow complexion and very slight jaundice, but not in all". In the discussion that followed the case report, Ghose, Coomer, Rakhaldas Ghose, Devendranath Dey, Bannerjee, Ahmed, Gibbons, Mcleod and Birch participated and the conclusions arrived at were as follows:—

(i) Infantile Cirrhosis of the liver was undoubtedly a peculiar disease found only amongst Indian children of the rich and the poor families alike.

(ii) The probable causative factors were the over-feeding of the children and bad hygenic conditions in which they were brought up.

(iii) The enlargement of the liver was probably due to either amyloid or fatty degeneration.

(iv) The pathology of the condition was doubtful and would remain so until the results of the careful post mortem examinations were available.

In a leading article on " Hepatic Cirrhosis in Children " in the February issue of the *Indian Medical Gazette* of 1888, the following observations have been made:—

" Is the disease, which has attracted attention in Calcutta, really Cirrhosis? If so, what is its cause? Syphilis, struma, malaria, faulty hygienic or dietetic error, or some other undiscovered and unsuspected factor? Does the disease occur elsewhere in Bengal or India? If so under what circumstances and conditions?.....

" The Calcutta Medical Society has pointed out a new and extensive interesting field of observation and study, and we trust that the observation will have the effect of attracting to it many workers and eliciting much light on what it must be confessed as still an obscure subject."

The next notable contribution to our knowledge of the disease is that of Gibbons (1890). The credit of recording the post-mortem findings of the liver in this disease for the first time must go to Gibbons who in his classical report on four cases has given a most graphic account of the anatomy of the liver. Green Armytage (1926) published an elaborate paper on " Infantile Cirrhosis of the Liver ", wherein he attributed the condition in addition to the general defective deficient nature of food " to the lack of essential salts and endocrines in cow's milk on which the children were fed ".

Considering the fact that the disease Infantile Cirrohosis is prevalent to a large extent in the Mysore State, the Government, impelled by their inherent desire to alleviate human suffering, created special facilities to carry out investigation regarding this disease. About 12 years ago Sankara Iyer of the Mysore Medical Service was specially deputed for conducting a survey on the prevalence of the disease in the State. In 1931 he published a report in which he mentioned that the distribution of the disease was confined to areas with a heavy rainfall; the worst affected parts being the districts of Kadur and Shimoga where the rainfall ranged from 80–145 inches and the less affected one being the Kolar District where the rainfall was less than 30 inches per year.

Tirumurti and Radhakrishna Rao (1934) investigated ' Infantile Biliary Cirrhosis ' cases and described three stages of the disease, *viz.*, (i) the stage of onset, (ii) the stage of enlargement of the liver, and (iii) the stage of contraction of the liver.

In a paper presented by me at the Mysore Medical Conference (1934) it was pointed out that *B. coli* was the probable causative factor for the production of cirrhosis. Subsequently in March (1935) at the next conference another paper was presented by me indicating that cow's milk and B. Coli were the ætiological factors and that the pathological condition of the liver was not that of biliary cirrhosis.

The next landmark in the field of literature on the subject is the contribution made by Radhakrishna Rao (1935). In a most interesting report on the "Histopathology of the Liver in Infantile Biliary Cirrhosis" he has described the morbid anatomy of the liver. He has maintained that the morbid condition of the liver is not that of biliary cirrhosis, but is a " subacute toxic cirrhosis ". The value of this contribution is all the greater for the reason that it is the second of the kind in which an elaborate account of the morbid condition of the liver has appeared after a lapse of 45 years.

Another noteworthy contribution is that of Lahiri (1936). He has shown that *B. coli* has a rôle in the ætiology of the disease, by isolating the organisms from the urine of the children suffering from the disease.

Balasundaram (1940) has published a series of three papers: the first of which deals with the distribution of "Infantile Biliary Cirrhosis in India", the second with the " Soil and Environment ", and the third with the " Diet and Habits of Children ".

Several other publications have appeared from time to time and all these deal with the clinical features and the ætiology of the disease. Excepting for one or two factors such as the occurrence of jaundice and the enlargement of the spleen, all the investigators are agreed as to the signs and symptoms of the disease, but with regard to the causative factors of the disease there is yet great difference of opinion.

In 1932 the task of conducting investigations regarding the disease was entrusted to me by the Government of Mysore. Since then, it was possible to study 105 cases, thanks to the co-operation of the medical men and women in the State who willingly referred the children suffering from this disease for my observation and treatment.

Male child Female child

FIG. (*a*) Twins

The female child being fed on cow's milk developed cirrhosis of the liver, whereas the male one being breast fed had no cirrhosis.

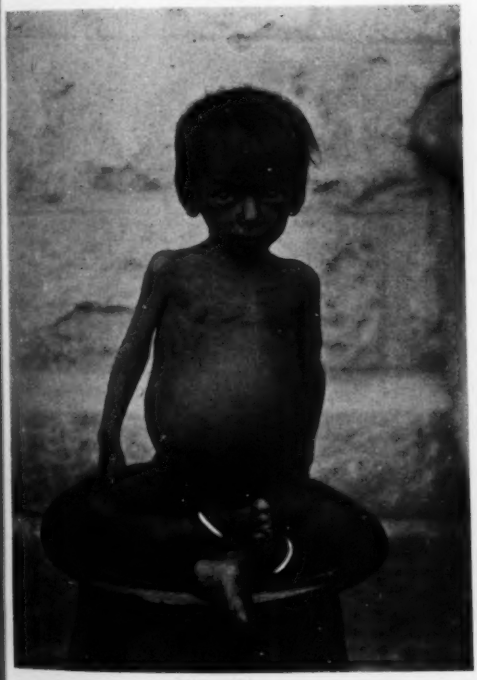

FIG. (*b*)

Advanced case of cirrhosis of the liver with ascites and œdema of hands and feet, but no jaundice.

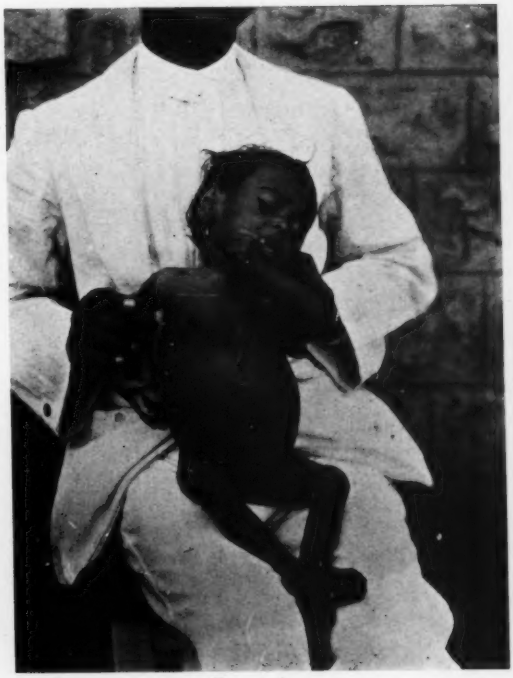

FIG. (*c*)

An advanced case of Infantile Cirrhosis with jaundice and in a state of cholæmia

FIG. 1

A portion of the anterior surface of the right lobe of the liver. Note the nodular surface of the liver (Case No. 1).

FIG. 2

Cut surface of the liver showing lobulations. The surface is rough and granular (Case No. 2).

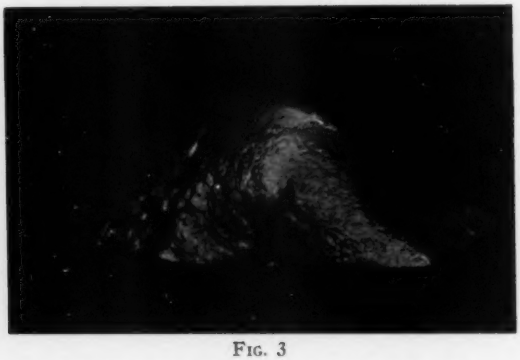

FIG. 3

Anterior surface of the entire liver (Case No. 4).

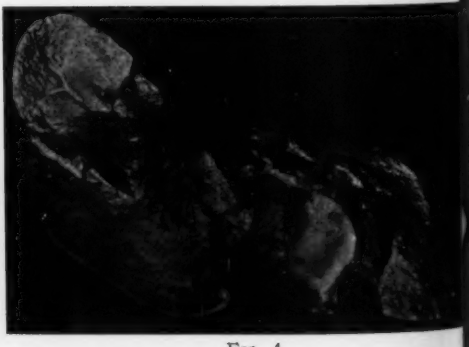

FIG. 4

Posterior surface of the liver (Case No. 4).

The present investigation was carried out on the following lines:— (1) The study of clinical features, (2) Laboratory tests, (3) Ætiological factors, and (4) Post-mortem and histological examinations.

In the present communication, a detailed account of the ætiology, pathology and morbid anatomy of the liver, is offered in the hope that it will lead to a clearer understanding of the many obscure features of the Infantile Cirrhosis of the Liver.

Clinical Features

The disease is peculiar to children between the ages of 6 and 24 months, and the onset is either insidious or traceable to an attack of fever, broncho-pneumonia or severe diarrhœa. The child is observed to lose appetite and to become irritable. There is distension of the abdomen and the mother's attention is drawn to the peculiar offensive odour of the urine voided by the child. The child suffers from constipation.

When the child is brought for examination and treatment the liver is observed to be enlarged, up to about two fingers' breadth, below the right costal margin; and the spleen is not palpable at this stage. The skin is dry and has a peculiar muddy appearance. In the course of 1 or 2 months after the onset of the disease, the liver is found to be enlarged up to the iliac crest on the right side, and up to about 2–3 fingers' breadth on the left side. On palpation it appears to be smooth and hard but not tender. Spleen may in some cases be found enlarged at this stage up to 1 or 2 fingers' breadth below the costal margin. In the course of another 2 or 3 months complications such as ascites, œdema of the hands and feet, puffiness of the eye-lids and face set in (Fig. *b*, Plate X). The abdominal veins become prominent due to the establishment of the collateral circulation. At this stage the liver is seen to be slightly contracted. Jaundice may develop in a few cases in the late stages of the disease. The accompanying chart will indicate that jaundice is not an invariable sign of the disease and it is found to be absent in some cases where the liver is enlarged even up to the iliac crest.

At the terminal stages of the disease which is about 8–10 months after the onset, there is gastro-intestinal hæmorrhage, and the child vomits digested blood (coffee-ground vomit) and passes dark-coloured stools. Cholæmia also occurs where jaundice has set in (Fig. *c*, Plate X). A few children die of intercurrent diseases such as broncho-pneumonia, diarrhœa or acute fever even in early stages as these children are easily susceptible to secondary infections.

Jaundice

Name	Age	Extent of liver enlargement	Jaundice	Ascites	Spleen
V.	1 year	3 fingers	Nil	Nil	Not palpable
S.	2 years	5 ,,	,,	,,	,,
B.	1¾ ,,	3 ,,	,,	,,	,,
S.	1½ ,,	3½ ,,	,,	,,	,,
S.	1½ ,,	4½ ,,	Slight tinge in the eyes	,,	,,
S.	2 ,,	4½ ,,	Nil	,,	,,
R.	1¼ ,,	2½ ,,	,,	,,	,,
R.	8/12 ,,	2 ,,	,,	,,	,,
K.	1¾ ,,	3 ,,	,,	,,	,,
G.	1¾ ,,	4 ,,	Slight	,,	,,
N.	2 ,,	2½ ,,	J+	+	Just palpable

Laboratory Findings

Examination of the blood—

Leucocytes.—The total leucocytic count varies from 8–14 thousands per c.mm. in the early stages of the disease. In the course of one or two months the number increases from 15–30 thousand per c.mm. At the terminal stages, the white blood cell counts may go upto 50 thousand per c.mm. Thus it is seen that leucocytosis is a marked feature of this disease. The differential leucocytic count shows a proportionate increase in the number of lymphocytes and the corresponding dimunition in the polymorphs. The lymphocytes vary from 35–70%, and the ploymorphs from 25–50%. The large mono-nuclears range from 1–3% and the eosinophils from 2–6%. There is no appreciable variation in the differential count of the leucocytes according to the different stages of the disease. The arneth count shows a definite shift to the left with an increase of monolobular polymorphs. The accompanying charts are given to show the various factors described above.

Leucocytic Count

Name	Total WBC	Poly.	Eosin.	Lymp.	Trsl.	L. Mons.
		Early Stages				
V.	10,800	33	4	58	2	3
R.	8,000	41	6	49	3	1
S.	11,000	56	2	36	2	4
S.	10,200	44	3	51	1	1
R.	6,000	62	2	31	3	2
Ch.	13,400	49	4	43	1	3
B.	10,000	24	5	68	1	2
N.	9,400	37	6	53	2	2
K.	12,200	40	4	54	1	1
T.	14,400	32	3	60	2	3
		Late Stages				
K.	15,000	42	4	48	2	4
R.	29,200	35	2	61	1	1
B.	33,000	28	5	64	1	2
G.	42,200	38	3	55	2	2
S.	18,600	45	7	40	4	4
S.	27,800	41	3	52	1	3
S.	34,300	52	2	39	4	3
R.	39,400	47	5	46	1	1
M.	48,100	36	4	57	1	2
V.	44,300	35	3	60	1	2

(Ten case records of each type have been given as typical examples).

The red blood corpuscles vary from 3–4 millions per c.mm. As the disease advances there is slight reduction in the number. The hæmoglobin varies from 50–70%. The blood picture shows a few poikilocytes and megalocytes but no nucleated red blood corpuscles. Microcytes predominate. On the whole it may be stated that anæmia is not a prominent feature of the disease.

Blood Smears—

Blood smears of amlost all the children have been taken from the finger tips and examined after staining them with Leishman and Geimsa stains. No parasites of malaria, kala azar or of relapsing fever have been found.

Blood Cultures—

Samples of blood were drawn under strict aseptic precautions from the cubital vein (in 15 cases) and inoculated into culture media such as bile-broth, nutrient broth and nutrient agar. No growth of any kind of bacteira was obtained.

Serological Tests

(1) *Vanden Bengh Reaction.*—Direct test is negative, and indirect reaction is normal. In jaundiced cases the test is direct positive or shows a biphasic reaction, depending upon the intensity of jaundice severe or mild.

(2) *Wassermann Reaction* is negative in all cases. (Bloods of parents have also been examined in almost all cases. Positive Wassermann reaction either in the child or its parents has disqualified the case being included in the list of Infantile Cirrhosis cases.)

Blood Sugar—

Blood sugar has been estimated in the following ten children (method used—MacLean's titration method). Blood was collected in the morning before any food was given, by pricking the finger, excepting in one case where it was drawn from the heart just after the death of the child.

Blood Sugar Table

Mgms. per 100 c.c. of Blood		Mgms. per 100 c.c. of Blood	
N.	47	K.	39
S.	38	B.	46
T.	42	S.	27 (Cadveric blood drawn from the heart)
H.	37	S.	34
R.	50	Ch.	30

It is noticed from the chart that blood sugar ranges from 0·03–0·05%. On the whole blood sugar is low and this is particularly so at the terminal stages of the disease. [The average blood sugar value for children, below the age of 2 years living in the Mysore State, as worked out by Srikantia (1938)

is 71 mgms. per cent., the lowest and highest figures being 63 and 100 mgms. respectively.]

Blood Urea—

In three cases blood urea was estimated and the following results were obtained:—

S. 18 mgms. per 100 c.c. (Cadveric Blood)
K. 15 „ „ (Early Stage)
M. 21 „ „ (Late Stages)

These 3 cases indicate that there is no abnormal increase of blood urea either at the early or terminal stages of the disease.

Stools—

In the early stages of the disease the stools are hard, scibulous and dark in colour. In the late stages the fæcal matter is whitish and of very offensive odour.

Microscopic Examination.—No ova or cysts of any parasite are found. There are no abnormal cells such as red blood corpuscles, macrophage, or epithelial cells found in the usual course of the disease; but when there is hæmorrhage blood corpuscles and leucocytes may be found. Fatty globules and fatty acid crystals are usually present in the fæces.

Cultures.—Cultures of fæces have been made in 50 cases. The following media were used:—Nutrient agar, blood agar, nutrient and bile broth, Endo's, Mackonkey's and Wilson Bleir culture media, were used at first and then the differentiating sugar media for isolating the bacteria. Growths of *B. coli-communior* and *B. coli-communis* were obtained in all cases and in a few others B. alkaligenes were also grown in addition. No pathogenic bacteria of the intestinal group such as *B. typhosus* or *B. dysenteriæ* were isolated. No growths of streptococci, either hæmolitic or non-hæmolitic, were obtained from these cultures.

Urine—

In the early stages of the disease the urine has the following characteristics:—Colour: Straw colour; Smell: A peculiar mildly offensive odour; Reaction: Acid; Specific Gravity: 1004–1012; Indican: Nil; Bile pigments and salts: Nil; Sugar and Albumin: Nil.

Microscopic Examination.—No casts or crystals of any kind are found. Here and there a pus cell may in some cases be found. There is often a large amount of amorphous phosphate present.

In the late stages of the disease, the colour of the urine is slightly yellowish in uncomplicated cases, and highly bile stained in jaundiced conditions. The peculiar smell is more intensive. Microscopic examination shows the presence of a few pus cells and epithelial cells.

Cultures of Urine.—Samples of urine were collected from male children in 20 cases under aseptic conditions and cultures put up. Only in 4 cases growths of *B. coli* were obtained and in one *B. alkalegenes*. The rest of the samples were all sterile.

Ascitic Fluid—

The ascitic fluid is usually straw coloured but may be tinged with bile when jaundice is present. The specific gravity varies from 1020–1030. It does not coagulate at room tempetature nor is there any cob-web formation within the fluid. Microscopic examination shows the presence of a few leucocytes, mostly lymphocytes, and very few red blood corpuscles. No bacteria of any kind is found. There are no cells of inflammatory reaction.

Chemical Tests.—There is only a trace of albumin and Rivalta's acetic acid test does not show flocculation. The fluid therefore is a transudate.

Animal Experiments—

5–10 c.c. of the ascitic fluid were injected into the peritoneal cavity of rabbits and guinæ-pigs. These animals did not show the signs and symptoms of peritonitis. One week later, they were dissected and there was no evidence of either local or general peritonitis or inflammatory reaction in any organ of the viscera.

Other Tests—

In one case, where post-mortem examination was conducted within half-an-hour after the death of the child, scrapings from the interior of the liver substance were taken, under strict aseptic precautions, by means of a platinum loop and inoculated into suitable media. Growths of *B. coli-communis* were found in all the media.

Morbid Anatomy and Histology

Material.—It is particularly to be mentioned that great difficulty is felt in getting bodies for post-mortem examination as the parents are most reluctant for handing over the dead children for post-mortem examination. Considering that very limited number of bodies are available for clinical autopsy even in the big hospitals of the State due to sentimental objections

FIG. 5

Low power view of the section of the liver; shows distinct lobulation of the liver—'multilobular variety' dilation of the portal veins and necrosis of the hepatic cells (Case No. 1). H. and E. Stain.

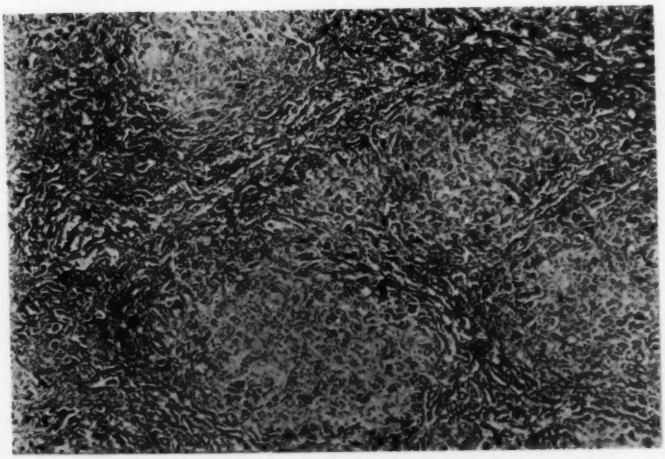

FIG. 6

High power view of the section of the liver. Formation of pseudolobulation and fibrous tissue, particularly at the portal spaces and distortion of the normal architecture of the lobules are clearly seen (Case No. 2). Mallory's Stain.

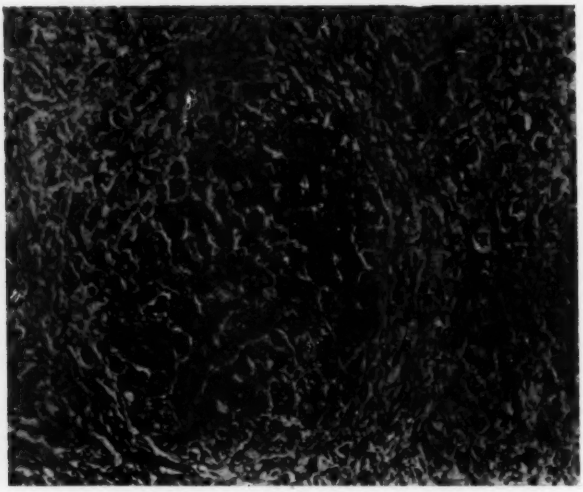

FIG. 7

A regenerating nodule in the liver tissue. Large cells with granular protoplasm. Note the bile capillaries at the periphery of the nodule are merging with the hepatic cells. H. & E. Stain.

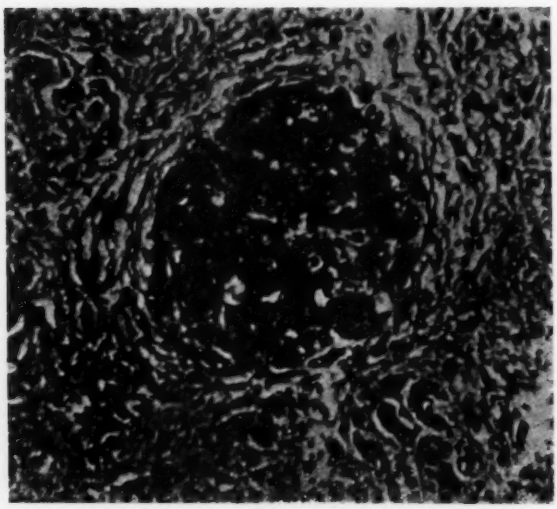

FIG. 8

Another regenerating nodule from Case No. 3. The nodule is big and surrounded by fibrous tissue. The cells in the neighbourhood are all necrosed. Mallory's Stain.

on the part of the parents, a study of 4 specimens of livers of 'Infantile Cirrhosis' cases is to be reckoned as sufficiently large to warrant a description of the pathological conditions of the liver associated with this disease. Permission was obtained from the parents to remove the liver in three cases; and in the fourth one bits of liver tissue were taken out.

Case No. 1 (1932).—Male child—1½ years old. Ill for 8 months with all the typical signs and symptoms of Infantile Cirrhosis; liver enlarged up the iliac crest on the right side—spleen just palpable—slight ascites—no jaundice—developed severe diarrhœa all on a sudden and died within 48 hours after—Gastric hæmorrage at the terminal stages.

Case No. 2 (1933).—Baby preserved at the Anatomy Museum, Medical College, Mysore, with an opening in the chest on account of dextrocardia.

Child had been admitted into the Victoria Hospital, Bangalore, for treatment of Infantile Cirrhosis in 1929—1 year and 10 months old—Had all symptoms of Cirrhosis—Liver enlarged upto 3 fingers' breadth—Intense jaundice and ascites were present—Died of Cholæmia. Father handed over the body to the Hospital authorities for preservation. Pieces of liver tissue were removed by permission, for investigation in 1933.

Case No. 3 (1936).—Child 2 years' old—female. Ill for 10 months—came under my observation 15 days before death. Had typical symptoms of Cirrohsis—Ascites—slight jaundice at the end—died of gastro-internal hæmorhage.

Case No. 4 (1940).—Female—age 1 year. Early case of Cirrhosis. Emaciated—week child—ill for 4 months—liver enlarged 2 fingers—spleen not palpable—no jaundice—no ascites. Comparatively early stage of Cirrhosis—developed broncho-pneumonia—died suddenly on the 8th day of fever.

Technique adopted.—Usually about 6–10 hours elapsed before the bodies were dissected, excepting in one case (No. 4) where within half-an-hour after the death of the child post-mortem examination was conducted. The organs were removed and fixed in 10% formalin for 24 hours and later on changed to a 4% solution. Frozen sections were prepared and stained by the following methods:—Sudan III for fat, and Rio-Hortiga's silver-stain for collagen fibres. Paraffin sections were stained by the following methods:—Hæmatoxylineosin stain for general examination and van Gieson and Mallory's stains for fibrous tissue and collagen fibres.

Naked Eye Appearance of the Liver—

The liver was found to be enlarged in all the cases. The measurements were as follows:—The greatest length from above downwards was 4–4½ inches, and the breadth from right to left 3½–4 inches. Anterio-posteriorly the right lobe measured from 2½–3 inches and the left from 2–2½ inches. The colour was reddish-brown in two cases and greenish in two others where jaundice had supervened. The surface was finely granular in all cases. In one case there were even small nodules of the size of pearl

barley particularly on the right lobe (Figs. 1, 3 and 4). The weight varied from 12–14 ounces. They were all hard and tough to cut and the cut-surfaces (Fig. 2) showed distinct lobulations.

Microscopic Appearances of the Liver—

Since all the 4 specimens show identical conditions it is found unnecessary to give a separate description of the livers in every case. A general account of the morbid histology is given in the following paragraphs, indicating the differences wherever they are found to be present:

A low power view of the liver shows the tissue to be divided into several uneven lobules (pseudo-lobule) by the formation of extensive bands of fibrous tissue. These lobules consist of hepatic cells which are in various stages of degeneration and necrosis (Fig. 5). Some of the cells contain brown pigmented granules but none of them shows fatty degeneration. Under high power, these hepatic cells are seen to contain granular protoplasm and a nucleus which is faintly stained or absent altogether. A few cells which appear to be normal and arranged in the form of cords or grouped in masses, may also be seen in some of the lobules. In none of these lobules the normal hepatic architecture is maintained (Fig. 6).

There are again, in certain portions of the liver, extensive areas of necrosis, where masses of cell debris surrounded by fibrous tissue, giving the appearance of small nodules are seen. The liver cells are big and contain nucleii which are rich in chromatin material.

Here and there are found big round or oval regenerating nodules (the so-called adenoma) consisting of a group of hepatic cells, surrounded by fibrous tissue. The hepatic cells within the nodules are large, contain granular protoplasm and a nucleus with well staining chromatin, and some of them are binucleate. The radicles of the hepatic vein are not seen within these nodules, but blood vessels occur at the periphery of the lobule in the fibrous band. The normal architecture of the hepatic parenchyma is lost in these regenerating nodules (Figs. 7 and 8).

The fibrous tissue is characterised by its irregular distribution, enclosing lobules of liver tissue without definite anatomical arrangement, giving the so-called ' multilobular ' appearance (Fig. 9). The silver impregnation preparation shows the manner of distribution of the collagen fibres. There is extensive formation of fibrous tissue—which is collagenous in nature—particularly in the portal space, and from here several branches appear to penetrate into the pseudo-lobules, to enclose cords of hepatic cells (Fig. 10)

FIG. 9

Low power view of the silver impregnated section.—Extensive formation of collagen fibres, particularly at the portal spaces (Case No. 1). Rio-Hortiga's Stain.

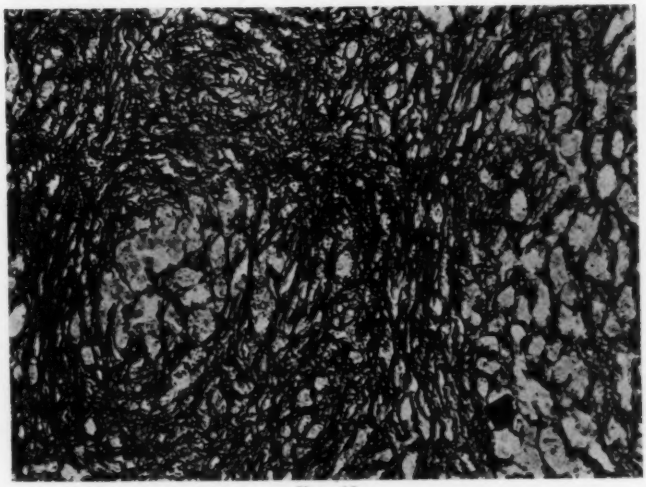

FIG. 10

High power view of the silver impregnated section. The collagen fibres are found within the lobules also, enclosing groups of cells. Rio-Hortiga's Stain.

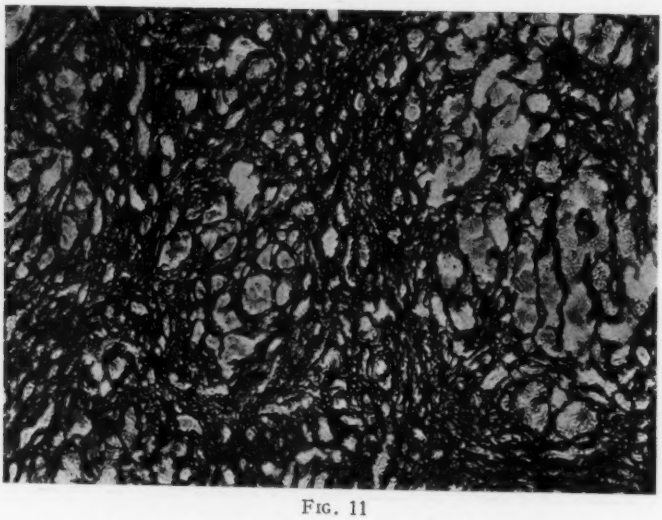

FIG. 11
Another view of the silver impregnated section.

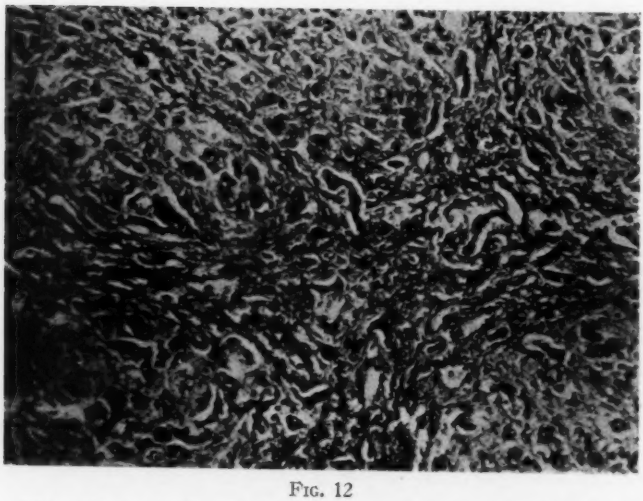

FIG. 12
Bile capillaries in the portal spaces. Extensive ramification of bile capillaries in the portal spaces. H & E. Stain.

In those areas where the regeneration of hepatic cells is less and the necrosis the most, the collagen fibres are found extensively, appearing in the form of whorls, enclosing smaller nodules of regenerating liver cells (Fig. 11).

The inter-obular bile capillaries have increased in number enormously. They ramify within the fibrous bands and their size varies according to the extent to which they are compressed by the rapidly growing fibrous tissue. The lining epithelial cells of these capillaries are cuboidal in shape and contain big nucleus. Some of these bile canaliculi are so much compressed by the fibrous tissue that they appear in the form of cords. The epithelial cells in some areas are seen to be communicating with strands of hepatic cells found either in the lobules or in the regenerating nodules, a feature propably indicating the origin of these cells (Fig. 12).

There is abundant infiltration of leucocytes both in the inter-obular bands of fibrous tissue and within the lobules. Polymorpho-nuclears, mono-nuclears, and lymphocytes are found and the latter are predominate. In the early stages of the disease (as in case No. 4) abundant infiltration of fibroblasts are seen in addition to the leucocytes.

In the specimen derived for an early case of Cirrhosis (case No. 4) the necrosis of cells and the formation of pseudo-lobulation are not so much advanced as in the other cases. The silver impregnation preparation, in this case shows delicate strands of collegen fibres to be more extensive at the portal spaces and branching off from there into the lobules.

The portal vein in the portal space is dilated (Figs. 5 and 9), and the smaller branches on the other hand are obliterated. The hepatic artery shows slight hypertrophy. The central veins, as such are not visible in the lobules.

Discussion

The disease "Infantile Cirrhosis" is discussed under the following categories:—(i) Ætiological factors, (ii) type of cirrhosis met with in the disease, and (iii) the manner in which cirrhosis of the liver is brought about in this disease.

1. Aetiological Factors

Fifty-one years ago Sen (1890) observed that "the ætiology of this fatal disease, more certain of its victims than even the dreaded cholera or small pox, is in darkness", and perhaps the same state of darkness regarding the knowledge of causation of this disease still persists! A study of the available literature on Infantile Cirrhosis reveals that various factors

have been considered to be the causative agents of the disease and these could be grouped as follows:—

(*a*) Infective agents,

(*b*) Food factors, and

(*c*) Other extraneous causes.

(*a*) *Infective Agents.*—

Malarial parasites, Leishman-Donovan bodies, intestinal worms and bacteria are the infective agents that are considered to be the probable causative factors.

Malaria.—It is generally believed that chronic malaria causes cirrhosis of the liver in some cases. Sen stated that the disease was not due to Malaria. Coomer (1887) in a "Comparative Study of Malaria and Infantile Cirrhosis" has stated that the signs and symptoms of this disease are quite unlike those found in children suffering from malaria. No author has recorded the presence of malarial parasites in the blood smears of infantile cirrhosis cases. My observation confirms that malarial parasites are not present either in the blood smears or those taken from the liver substance.

Thomson and Robertson (1929) observe that " Cirrhosis of the liver due to malarial infection has been frequently described, but other authorities are of the opinion that this is rarely caused by malaria *per se*, resulting as a rule from some other concomitant disease ". Manson-Bahr (1940) emphasises that malaria has " nothing to do " with this disease.

Kala-azar.—Castallani and Chalmer (1919) doubted whether infantile cirrhosis was not a variety of Kala-azar. Several blood smears from scrapings of liver have been examined and I have not detected the presence of Leishman-Donovan bodies even in a single case. So far as I am aware Kala-azar is not prevalent in the Mysore State.

Intestinal Worms.—Animal parasites such as Ascaris, Ankylostoma, and the cestodes cannot be stated to be the causative agents since either the parasites or the ova have not been recorded from the examination of the fæces of the children suffering from " Infantile Cirrhosis ".

Bacterial Infection.—Bacterial infection is one of the many accepted causes of cirrhosis in general. So far as Infantile Cirrhosis is concerned there are reports to show that *B. coli* and *streptococci* have been recovered from the urine.

In 1934 it was reported by me that *B. coli* were isolated from the urine in two cases of Infantile Cirrhosis.

Lahiri (1936) obtained growth of *B. coli* in one case and *streptococcus hæmolyticus* in another, from urine, out of a total of 11 cases. He is of the opinion that failure to get growth of *B. coli* in most of the cases may be due to the fact that the bacteria are excreted in showers with negative phases in the interval.

Manson-Bahr (1940) states that 'Megaw has pointed out recently the close association between the cirrhosis of the liver and bacillary dysentery. He believes the one to be the direct sequel of the other'.

In the present investigation, it has been pointed out that *B. coli* was isolated from the urine and also growths of colonies of *B. coli-communis* were obtained from cultures of scrapings of the liver.

It has been suggested that the "so-called positive bacterial findings are due to secondary invasions" after death (Bhattacharjee, 1933). Moon (1931) obtained growth of *hæmolytic streptococci* from the liver of a child with portal cirrhosis at the post-mortem examination whereas a blood culture made a few hours before death was negative indicating that the presence of bacteria in the liver was not incidental to general septicæmia.

In order to verify whether the presence of *bacteria* in the liver is merely due to mechanical invasion after death, a number of cultures were put from the liver substances taken from various kinds of cadavers available for the post-mortem examination, such as accidental deaths, drowning, hanging, septicæmia, chronic suppative condition terminating in pyæmia, etc., in 20 cases. There was no growth of any kind of bacteria obtained even in a single instance. Even in a case of pyæmia, resulting from osteomyelitis due to staphylococcal infection, where small abscess were found in the liver as well in other organs, growths of *staphylococci* were obtained only from the abscess in the liver, but neither *staphylococci* nor *B. coli* were grown from the scrapings taken from a healthy portion of the same liver. These investigations indicate that the presence of bacteria in the liver is not due to accidental or mechanical invasion of the organ by the organisms after death.

(*b*) *Food Factors.*—

(i) *Milk.*—Cow's milk; breast milk; goat's milk, etc.

Majority of the authors are of the opinion that defective diet is responsible in producing the disease of the liver in children. Ghose (1887) was of the opinion that "children who were never put to mother's breast and fed from birth either with milk of the cow, ass or goat, or by different kinds of infant food, suffered from this disease; excepting those that were brought up by a healthy wet nurse." Rakhaldas Ghose (1887)

maintained that "for 6, 7 or 8 months infants get nothing but milk and that without consideration of interval, as often in fact as they cry for it." Dey (1887) on the contrary thought that overfeeding of children with breast milk was the cause of the disease. Roy (1887) did not agree with Dey's opinion and asserted that by stopping mother's milk and by substituting other milks, he had not prevented the occurence of the disease. Green Armytage (1926) who had studied a large number of cases—63 in all—stated that children overfed on foods which had very little nutritive value and particularly cow's milk which was lacking in vitamins, developed cirrhosis of the liver. Krishna Ayyar (1927) was of the opinion that by the use of tinned food and cow's milk this disease was brought about. Gothoskar (1931) observed that the disease occurred very rarely in children who were exclusively breast-fed. He thought that children fed with cow's milk suffered from the disease on account of low contents of fat.

(ii) *Other Food Factors.*—Fat, sugar, etc.

Ahmed (1887) considered that children fed on "farinaceous food" suffered from the disease and he favoured milk as the best suited food for children.

Vaidyanatha Iyer (1926) thought that the disease was due to over-feeding of children with unsuitable and undigestible food-stuff.

(*c*) *Other Extraneous Causes.*—

(i) *Racial and Social Status.*—J. N. Ghose (1887) observed that "children of the town as well as of the country, rich and poor equally suffered from this malady and that he had not seen many cases amongst the Muhammadan and European population." Rakhaldas Ghose (1887) stated that the "disease was confined to the children of the rich and the middle classes only, and he had not met with the peculiar enlargement under discussion, amongst poor people. Ahmed (1887) remarked "that the children of the rich and the poor were equally liable." Mcleod (1887) observed that he had not seen any case of this enlargement of the liver in a European child. Green Armytage (1926) stated that in Calcutta children suffering from this disease were found amongst the European, Anglo-Indian, Hindu and a few Muhammadans. Vaidyanatha Iyer (1926) stated that the disease was prevalent amongst vegetarians and non-vegetarians. Sankara Iyer (1931) reported that 90 per cent. of the cases were amongst the Brahmins and the disease was common in families of middle and lower classes.

(ii) *Altitutde, Multiple-pregnancies, etc.*—Mukherjee (1922) stated that this disease was not found in places at a higher altitude than 1,000 feet above the sea-level.

Sankara Iyer (1931) observed that quick succession of preganancies to be a predisposing factor in producing the disease in children.

Conclusion.—

A study of a large number of Infantile Cirrhosis cases in the Mysore State has led me to the following irrestible conclusions regarding the ætiological factors of the disease:—

(i) The disease is met with in the rich as well as the poor families. Male and female children are equally susceptible.

(ii) Majority of the children belong to the vegetarian (98 per cent.) and a few (2 per cent.) to the non-vegetarian families. Not a single case has been observed from amongst the Mussalman, European or Anglo-Indian families.

(iii) All the children that came under my observation, suffering from Infantile Cirrhosis were either entirely or to a large extent fed on cow's milk from birth. There was either no supply of mother's milk or when present was very scanty.

(iv) Not a single case of Infantile Cirrhosis has been observed in children that were entirely fed on breast milk in sufficient quantity.

(v) Increase or decrease of fat or sugar in the dietary has not prevented the occurrence of the disease in children.

(vi) Substitution of modern patent 'Infant-milk-foods' for cow's milk has prevented the occurrence of the disease in Infantile Cirrhosis families.

(vii) Altitude has no bearing in the production of the disease. Mukerjee's statement that the disease is not prevalent in places higher than 1,000 feet above the sea-level is not tenable because in the Mysore State there is no place that is below 2,000 feet from the sea-level (*Mysore Gazetteer*), yet Infantile Cirrhosis is prevalent to a large extent.

(viii) Repeated pregnancies of mothers do not form a predisposing factor in the production of this disease in children. 68 per cent. of my cases were either the first or the second born ones, 18 per cent. the third born ones, and the remaining very few cases were from mothers who had borne a large number of children.

(ix) Bacillary dysentery has no ætiological rôle in bringing about cirrhosis of the liver. None of the children under my investigation suffered from bacillary dysentery.

I am of the opinion that there are two important factors in the ætiology of the disease. One is cow's milk and the other is the invasion of

B. coli into the liver. Cow's milk brings about the predisposing factors of the disease—gastro-intestinal disorder and devitalization of the liver—and *B. coli* completes the pathological process.

Conclusion.—

The following case that came under my observation emphatically suggests that cow's milk is an important factor in the causative rôle of the disease. A Hindu woman gave birth to twins—one a male and the other a female child (Plate X, Fig. *a*). As she was not strong enough to feed both the children on breast milk she thought of feeding one of them only on her own milk and the choice fell on the male child ! The female child which was fed on cow's milk developed cirrhosis of the liver in her eighth month. The male child was entirely free from the disease even though he was suffering from malignant tertian malaria at the time. It is noteworthy to observe that these two children brought up in identical environment but fed on different kinds of milk reacted differently with regard to the development of cirrhosis of the liver. This is an unique illustration which supports the view expressed above. (Reported at the Mysore Medical Conference, 1935.)

2. The Type of the Cirrhosis found in this Disease

It is a well-known fact that the classification of cirrhosis of the liver is a difficult problem. It is also recognised that the classification based on either purely anatomical changes or on clinical findings alone would lead to an unlimited and confusing number of varieties of cirrhosis. A satisfactory, though not ideal, classification, would be that which is based on the combined findings of morbid anatomy and certain characteristic clinical conditions.

While discussing the nature of cirrhosis found in this disease, one has to take into consideration the characteristic features of the well-recognised types of cirrhosis of liver and compare those with the findings met with in Infanitile Cirrhosis. The most usual types of cirrhosis that require consideration are the following:—

(i) Portal or Lænec's cirrhosis, (ii) Biliary cirrhosis, (iii) Hanot's cirrhosis, (iv) Cognenital syphilitic cirrhosis and (v) Cirrhosis in Banti's, and (vi) Wilson's Diseases.

The three latter diseases are peculiar to children and they require consideration at first. According to Pearson and Wyllie (1935) and other authors in congenital syphilis the liver shows a pericellular type of fibrosis and the disease is associated with signs of skin lesions and enlargement of the spleen. A blood examination shows positive Wassermann reaction. In

Banti's disease, there is a huge enlargement of the spleen and liver cirrhosis is secondary to that of splenic enlargement. Cirrhosis in Wilson's disease is also a multilobular variety but the disease is characterised by the progressive lenticular degeneration. Therefore these three types of cirrhosis may be left out of consideration in connection with Infantile Cirrhosis. Hanot's hypertrophic biliary cirrhosis is a condition in which there is marked jaundice from the beginning of the disease without the formation of ascites. Section of the liver shows marked fibrosis, dividing the tissue into little groups of cells. The clinical condition of the disease and the histological features of liver in Infantile Cirrhosis do not conform with Hanot's types.

Biliary Cirrhosis.—The liver in biliary cirrhosis has the following characteristic features:—

(1) It is large, smooth and intensely stained with bile.

(2) Microscopically the fibrous tissue, which is not large, is usually found around large bile ducts every lobule of the liver forming a unilobular type of cirrhosis. Liver cells do not show great degree of degeneration. There is relatively little evidence of extensive loss of liver tissue.

(3) Clinically there is jaundice as a result of mechanical obstruction of the bile passage without the development of ascites.

Portal or Lænnec's Cirrhosis.—

Criteria.—While discussing the criteria of the cirrhosis of the liver, Moon (1934) in his review on "Experimental Cirrhosis in Relation to Human Cirrhosis" observed that "the conception that 'Cirrhosis' is synonymous with 'Chronic diffuse hepatites' is generally accepted. He further stated that "any agent or combination of agents which produce chronic diffuse hepatic inflammation will result in some degree of cirrhosis. The degree and character of this may vary but the essential features of the cirrhotic process are (i) degeneration and destruction of liver cells, (ii) regeneration of liver cells from those which escape destruction and (iii) proliferation of connective tissue.

"In portal cirrhosis in advanced stages there is marked disturbance of the portal circulation. In biliary cirrhosis there is no obstruction to the portal circulation or obliteration of hepatic architecture."

Moon further summarises the views of various authors such as Kertz, Kelly, McNee, Gohn, MacCallum Mann, *et al.* regarding the criteria of portal cirrhosis in the following manner:—

"Lænnec's Cirrhosis is the result of attacks of degeneration and destruction of liver cells, that the entire parenchyma has been totally renewed

several times and this results in complete architectural transformation of the parenchyma (Kertz.) The chief characteristic of Lænnec's Cirrhosisis is complete disarrangement of hepatic architecture. The regenerated nodules do not contain radicles of the hepatic vein (Kelly). The architectural alteration with complete disarrangement of the circulation is a prominent feature of the Lænnec's Cirrhosis. (McNee, Gohn)." He is also of the opinion that " portal cirrhosis in children is not unusual and that it occasionally affects more than one in the same family."

The liver in Infantile Cirrhosis, as described already, has the following characteristics:—

(1) It is enlarged, has a fine granular surface and is hard to the touch.

(2) Microscopic appearance is characterised by the formation of fibrous tissue which is distributed in a disorderly manner giving the " multi-lobular appearance ". Within the lobules the liver cells are found in various stages of destruction, and hepatic veins are not visible in the centre. The destruction of liver cells in certain areas is so complete that there are only masses of necrosed cellular debris surrounded by fibrous tissue.

Regenerating nodules are found and they contain big hepatic cells which are not arranged in the normal radiating manner but grouped in masses.

(3) Clinically there is development of ascites at the terminal stages of the disease. Jaundice is not present in all cases, but occurs in a few cases at the late stages of the disease.

The histological features of the liver and the clinical conditions of the disease satisfy all the criteria of portal cirrhosis and '*Infantile Cirrhosis*' is, therefore, undoubtedly a case of *portal (or Lænnec's) Cirrhosis.*

Gibbons (1890) on the other hand states that he has " no hesitation in calling this as " Biliary Cirrhosis " and attributes the following reasons for classifying the liver as such:—

" (1) The surface of the liver is not hob-nailed and rough. It is smooth, finely granular and resembles morocco leather.

" (2) The liver substance is deeply bile-stained, a condition not usually found in portal cirrhosis.

" (3) The fibrous tissue is not confined to the interlobular spaces but is found within the lobules separating the hepatic cells each of which may be surrounded by fibres or groups of two, three, or more may be found encircled by one band.

"(4) The most striking feature of the microscopical appearance of these livers is the number of bile ducts which ramify in the newly formed fibrous tissue. Most of these bile ducts are of new formation, others are the pre-existing interlobular bile ducts.

"(5) The liver tract in many parts is completely destroyed and replaced by fibrous tissue. The liver cells show marked destructive agents. In the greater part of the section they are absolutely destroyed and transformed into shapeless mass of granular debris, pigment granules and minute oily globules.

"Here and there in the section a few small masses occasionally a lobule, may be found the cells of which are nearly normal, though rather small. A few cells have two nuclei. It is astonishing how few cells presenting at all normal aspect can be discovered in one section of these livers.

"(6) The size of the lobules varies. The hepatic cells are nowhere arranged in regular radiating rows as in normal liver and they are encircled by broad bands of fibrous tissue. Under high power the fibrous tissue is seen not only around the lobules but also between hepatic cells. The destruction of hepatic cells is most complete in these parts of the section in which their development of fibrous tissue around the cells is greatest. The lobules are found in every stage of destruction upto complete disorganisation.

"(7) In many cases of portal cirrhosis the liver is enlarged through the whole course of the disease and does not undergo the usual contraction and on the other hand the liver in biliray cirrhosis may become diminished in size."

It is worthwhile examining at some length the argument put forward by Gibbons to diagnose the morbid condition of the liver as biliary cirrhosis. First of all Gibbons stated "that the surface of the liver should be hobnailed and rough if there should be portal cirrhosis." This is not necessary. "The surface of the liver may be smooth or finely granular and may even be bile stained in portal cirrhosis (Delafield and Prudden, 1936).

Secondly, the ramification of bile ducts within the newly formed fibrous tissue is considered to be the most striking feature of biliary cirrhosis by Gibbons. In portal cirrhosis according to Delafield and Prudden (1936), Bell (1938), MacCallum (1938) and Muir (1936) the bile ducts are more numerous than normal and at times they are more prominent than the connective tissue. The bile ducts are seen to branch and occasionally these branches are definitely connected with the hepatic cords. Therefore

ramification of bile ducts cannot be considered to be the distinguishing feature of biliary cirrhosis.

Thirdly Gibbons emphasised that extensive destruction of the liver cells and the loss of radiating arrangement of these cells within the lobules to be the characteristic feature of biliary cirrhosis. It has been pointed out already according to Kertz, Kelly, McNee, Gohn, Moon, and other authors that the destruction of liver cells, loss of normal architecture of the lobules and the extensive formation of fibrous bands in portal areas are the chief criteria of portal cirrhosis and not of biliary cirrhosis.

Fourthly, MacCallum (1938) states that in biliary cirrhosis " there is no great departure from the regular lobulation and such extreme changes as seen in diffuse nodular cirrhosis are rarely found in biliary cirrhosis."

Fifthly Gibbons has noted the presence of masses of liver cells surrounded by fibrous tissue resembling a lobule, in the midst of degenerating tissue and also states that there were two nuclei in some of those cells. Evidently these masses of cells are no other than the regenerating nodules which are so characteristically found in portal cirrhosis and not in biliary cirrhosis.

It is necessary to emphasize that according to his own description, Gibbons was dealing with livers of 'Portal Cirrhosis' and not of ' biliary cirrhosis '.

Sen (1890) pointed out in the beginning alone at a meeting subsequent to that in which Gibbons read his paper, " that the specimens described by Gibbons showed most marked degeneration and destruction beyond recognition of the liver cells and this from the pathological point of view, might be considered a fatal obstacle to the disease being grouped under the head of hypertropic biliary cirrhosis".

In a brief report on the disease I pointed out in March 1935 that the histological picture presented by the liver was far from being that of biliary cirrhosis.

Radhakrishna Rao (1935) also states that it is not ' biliary cirrhosis ' that is found in this disease.

Sub-acute Toxic Cirrhosis.—In an exhaustive description of the histopathology of the liver, with numerous illustrations, Radhakrishna Rao (1935) designates the morbid condition of the liver as one of ' sub-acute toxic cirrhosis ' and attributes the following features to be the criteria of that type of cirrhosis:—

(i) The presence of avascular non-inflammatory odematous connective tissue network, enclosing in its meshes small-sized islands of hepatic parenchyma of very unequal size, (ii) obliterative lesion of the terminal and some of the bigger divisions of the hepatic venous tree, (iii) areas showing disorganisation of the reticulum of the sinusoidal capillary bed mostly around the hepatic venous terminal, (iv) poor attempt at regeneration of the hepatic parenchyma, (v) the presence of very small pseudo-lobules compared to those usually seen in the portal cirrhosis and the poor attempt at the regeneration of the hepatic parenchyma, as evidenced by the small size of the pseudo-lobules and (vi) the presence of varying degree of necrosis of the liver cells almost uniformly distributed throughout the organ.

He also considers that some kind of a toxin brings about the primary lesion of the hepatic veins and the parenchymal loss in the liver tissue is only secondary. The action of the toxin is not acute but probably sub-acute resulting in retardation of regeneration and continuous necrosis of the hepatic parenchyma.

It is to be pointed out that the above-mentioned arguments of Radhakrishna Rao do not warrant the designation of a new type of cirrhosis. If the primary lesion is in the hepatic venous tree as stated by him due to 'Phlebosclerosis, endophlebitis and partial thrombosis' the effects of such an obliteration on the liver tissue would be different from what has been observed in the livers of these children. McNee (1932) who has made an extensive study of the intricate vascular arrangement of the liver is of the opinion that the result of obstruction of efferent veins is not that of fibrosis but of necrosis of the cells of the centre of the lobules. He (McNee) further quotes the experimental works of Bolton (1914), and of Bolton and Barnard (1931) on the effect of artificial obstruction to the efferent vessels of the liver, and states that " as a result of partial obstruction of the inferior venacava in monkeys and cats there was widespread degeneration of the liver parenchyma especially towards the centre of the lobules, leaving islets of intact cells around the portal canals. The necrotic cells soon disappeared, their place being taken by dilated sinusoides, no trace of fibrosis ever being observed. The absence of fibrosis in all those experiments which involved obstruction to one or other part of the blood supply of the liver, is a point of very great importance ". It is therefore unlikely that the primary lesion in Infantile Cirrhosis is in the hepatic venous tree.

The second aspect of Radhakrishna Rao's argument has been that the lesion in Infantile Cirrhosis is not ' portal cirrhosis ' for the reason that there are very small pseudo-lobules and the attempt at regeneration is poor. This argument in my opinion is also untenable. MacCallum (1938) has described

the process of the formation of nodular (portal or Lænnec's Cirrhosis) cirrhosis in the following lines:—

"The liver cells are killed in patches—whole lobules and groups of lobules at a time or only parts of lobules. There remain irregular masses of liver tissues, partly disconnected from bile ducts. The frame-work of the rest of the tissue collapses and shrinks, and is kept in that position by the growth of new fibrous tissues. The bile ducts which were interrupted by the death of the liver cells send out sprouts which attempt to connect again with liver-cell strands. For a time the liver cells are normal, but the whole process may be repeated, not only once, but many times. It is clear that this must lead to an extraordinary distortion of the liver structures. There are no larger lobules, but only nodules, produced by hyperplasia of smaller groups of cells which were left intact. In portal cirrhosis there may be either pseudo-lobulation or merely nodules of liver cells particularly in late stages of the disease."

The absence of large pseudo-lobulation in the liver does not exclude portal cirrhosis and there is distinct and liberal attempt at regeneration of the hepatic parenchyma in the livers of ' Infantile Cirrhosis '.

Thirdly the designation ' sub-acute toxic cirrhosis ' would suggest the causative factor of the disease to be a ' toxin ' which has not been established. Moreover the term ' sub-acute ' does not signify any degree of the intensity of the ' processs ' peculiar to the livers of Infantile Cirrhosis cases only; for every kind of Cirrhosis is considered to be the result of a chronic, or low grade pathological reaction.

Nomenclature of the Disease

B. C. Sen (1887) who reported the disease for the first time, has designated the condition as ' Infantile Cirrhosis ' and Green Armytage (1926) also called the disease by the same nomenclature. To say that this disease is 'Infantile Biliary Cirrhosis' would imply that the causative factor of Cirrhosis is obstuction to the bile passage, and the pathological condition is that of biliary cirrhosis, which as has been pointed out, is not the correct state of pathological conditions. It is suggested that the nomenclature of the disease should be " Infantile Cirrhosis " and not " Infantile Biliary Cirrhosis ".

3. The Manner in which Portal Cirrhosis is brought about in this Diseaese and the Causative Factor

The problem of the development of liver cirrhosis is a vexed question. In recent times Rossle (1930) has explained the problem in a convincing

manner. First of all he has tried to limit the conception of cirrhosis of the liver by advancing the view that the development of the connective tissue around the perivascular tissue without any kind of damage to the liver cells should not be considered as true cirrhosis. Secondly he has emphasised the fact that mere damage to the liver parenchymal cells without the destruction of the connective tissue would not lead to the development of cirrhosis, but only to the regeneration of the hepatic cells.

According to Rossle one must differentiate between the pure outgrowth of the peri-portal connective tissue, without damage to the liver cells which would occur in cases of stasis of bile in the capillaries and in collengitis and the true cirrhosis which arises as a result of damage to the parenchymal cells as well as the fine connective tissue.

McNee (1932) on the other hand considers that in cirrhosis, "the formation of fibrosis is neither the result of a primary disease of the supporting connective tissue, *i.e.*, a true chronic interstitial hepatitis with secondary changes in the glandular cells; nor the lesion just the reverse—a primary atrophy or degeneration of the hepatic glandular cells followed by a replacement fibrosis". He puts forward the following as his views regarding the evolution of the lesion in cirrhosis hepatis:—

"The primary lesion consists in degeneration or destruction of liver cells, followed by regeneration and replacement by new liver cells, but with some overgrowth of fibrous tissue in the areas which are destroyed. Areas of liver cells die and are replaced by new areas built up from surviving cells; these new areas are again destroyed and are again rebuilt, but increase of fibrous tissue gradually supervenes as the process of death and repair is repeatedly carried out. Finally, on this view, an entirely new liver parenchyma, containing perhaps not a trace of the old and original liver, is built up on a plan which has the appearance we described as cirrhosis. Even the blood vessels and their branches—both of the hepatic artery and of the portal vein—are involved, and the whole circulatory plan in the liver is markedly altered."

In 'Infantile Cirrhosis' as has been pointed out already, there is unmistakable evidence of repeated destruction and regeneration of hepatic cells, and the development of fibrous tissue. There is presumably some agent (an infective) setting up a chronic inflammatory process in the liver substance, resulting in repeated destruction and regeneration of the liver cells and the ultimate production of true cirrhosis.

The Causative Factor :—

The question that naturally arises is as to the nature of the substance that causes the inflammatory process. In order to find an answer, one has to look into the literature available on the experimental production of cirrhosis and correlate those conditions with the cirrhotic processes found in the liver of the children. A vast number and variety of substances have been used experimentally for the production of cirrhosis and they could be grouped as follows:—

(i) Metallic substances,

(ii) Organic and inorganic chemicals,

(iii) Infective agents such as bacteria.

Heavy metals such as copper and manganese [Malloy (1921), Parker and Ney; Findley, Hurst and Hurst (1928), Krishna Rao (1931), Shindle (1931), and Albot] have been used in order to produce cirrhosis of the liver in animals—but the results obtained are not uniform with all the experimenters. It cannot therefore be claimed for certain that these metals produce cirrhosis of the liver.

Organic substances like tar, shale oil and carbon tetrachloride have been successfully used by a large number of experimenters to produce portal cirrhosis. But these materials have no ætiological factor in the causation of the disease is borne out by the fact that children do not come in contact with these substances in whatever manner.

Several kinds of bacteria have been used to produce experimentally either degeneration and necrosis or cirrhosis of the liver, and from out of these I mention here particularly those that have produced cirrhosis of the portal type. They are *B. coli*, *streptococci* and *B. tuberculosis*.

B. coli *and Cirrhosis of the Liver.*—

As early as 1898 Adami, while discussing the " Etiology of progressive hepatic cirrhosis", came to the conclusion that there was a direct connection between the presence of bacilli in the liver of cirrhosis and the process". His attention to the examination of cirrhotic livers for the presence of *B. coli* was drawn from the constant discovery of these bacilli in animals afflicted with *Pictou* cattle disease (a disease characterised by a peculiar extensive cirrhosis of the liver). Opie (1910) (quoted by Moon) successfully produced cirrhosis in dogs by injecting chloroform at first (to bring about degenerated condition of the organ) and later on *B. coli* Lahiri (1936)

also produced cirrhosis of the liver in dogs by feeding them at first on excess of fat for sometime and when the liver had undergone slight degeneration, by injecting *B. coli* in successive doses.

Infantile Cirrhosis and B. coli.—

From an analogy of the experimental work on the production of cirrhosis of the liver on the one hand and the discovery of *B. coli* in the liver of an Infantile Cirrhosis case on the other, I am of the opinion that *B. coli* is the infective agent that is responsible to bring about the pathological process in the children suffering from this disease. Neither tubercle bacilli nor *streptococci* appears to be the causative agent for the reason that the microscopic examination of the liver has not shown the presence of tuberculous lesion and smears from liver substance have not shown the presence of either of these bacteria.

B. coli which is the normal inhabitant of the intestine becomes pathogenic due to the changes in the intestinal environment and gains access to the liver which is already devitalized and sets up a chronic inflammatory process. I conclude the discussion by adducing the following evidence in support of my view that *B. coli* is the causative agent in bringing about the cirrhotic process in disease of the children.

(i) Smears of liver substance have shown the presence of gram negative bacilli morphologically identical with *B. coli.*

(ii) Cultures made out of the scrapings of the liver substance have yielded the growth of *B. coli-communis.*

(iii) Cultures of urine in four cases have shown the growth of *B. coli.*

(iv) Vaccine prepared out of *B. coli* grown from the fæces of children suffering from Infantile Cirrhosis and inoculated in successive increasing doses over a period of 6 to 7 weeks has cured the disease. (This fact indirectly proves that *B. coli* to be the infective agent in the production of the disease.)

Summary

(1) Infantile Cirrhosis is a disease found to a greater extent amongst the children of the vegetarian and to a lesser extent in the non-vegetarian families. The disease is not met with amongst the Muslims and the Europeans.

(2) There are two factors in the ætiology of the disease; cow's milk and *B. coli* infection. Cow's milk brings about the predisposing factor—

viz., gastro-intestinal disorder and devitalization of the liver—and *B. coli* the cirrhotic changes in the liver.

(3) The pathological condition of the blood indicates an increase of leucocytes with lymphocytes predominating. The blood sugar is low and blood urea is normal.

(4) Jaundice is present in a few cases only and this complication arises as a result of obstruction to bile capillaries in later stages of the disease.

(5) The morbid anatomy of the liver presents a picture of *portal cirrhosis*. The clinical condition of the disease as well as the histological features of the liver fulfil the criteria of portal or Lænnec's cirrhosis.

(6) It is suggested that the nomenclature of the disease should be 'Infantile Cirrhosis' and not 'Infantile biliary Cirrhosis'.

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SUBULURA MINETTI N. SP. (NEMATODA) FROM AN INDIAN FOWL*

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Some nematodes from the intestine of a fowl from Jaipur were received last year at this Institute. These on examination proved new to science.

The worms are cylindrical, the males having a curved posterior end. The head is provided with two lateral, two sub-median dorsal and two sub-median ventral papillæ. The mouth is hexagonal and has distinct lips. The mouth capsule has thick chitinous walls and is enlarged posteriorly, where it has three small teeth. The lateral alæ extend backwards as far as the hinder level of the œsophageal bulb. The œsophagus gradually thickens posteriorly and is followed by a distinct, almost spherical bulb. The intestine is enlarged anteriorly.

The male measures 6·65–12·6 mm. in length and 0·272–0·4 mm. in maximum breadth, which is attained slightly behind the œsophageal region. The buccal capsule measures 0·028 × 0·018 mm. The œsophagus is 0·83–0·95 mm. long. The œsophageal bulb measures 0·18–0·23 mm. laterally. The anus is situated at 0·246–0·276 mm. from the posterior extremity. The pre-anal sucker is fusiform, without a chitinous wall, and measures 0·182–0·277 mm. antero-posteriorly. The spicules are equal and measure 1·085–1·17 mm. in length. The gubernaculum has a spur anteriorly and measures 0·169–0·223 mm. in length. There are ten pairs of caudal papillæ of which three pairs are pre-anal, two are ad-anal and five post-anal.

The female measures 10·2–15·35 mm. in length and 0·35–0·502 mm. in maximum thickness. The buccal capsule measures 0·029–0·04 × 0·002–0·034 mm. The œsophagus is 0·925–1·13 mm. long. The œsophageal bulb measures 0·27 –0·303 mm. laterally. The nerve ring and the excretory pore are situated 0·17–0·35 mm. and 0·457–0·527 mm. respectively from the anterior end. The tail is 0·72–0·8 mm. long. The vulva is situated on a slight prominence 4·4–5·9 mm. from the anterior end of the body, approximately at the anterior two- to three-sevenths. The ovijector, measuring 0·386 mm. in the specimen 10·2 mm. long, is directed anteriorly. It consists of a muscular portion which enlarges proximally and forms a cup-shaped cavity, in which is enclosed a "circular hyaline area" as described

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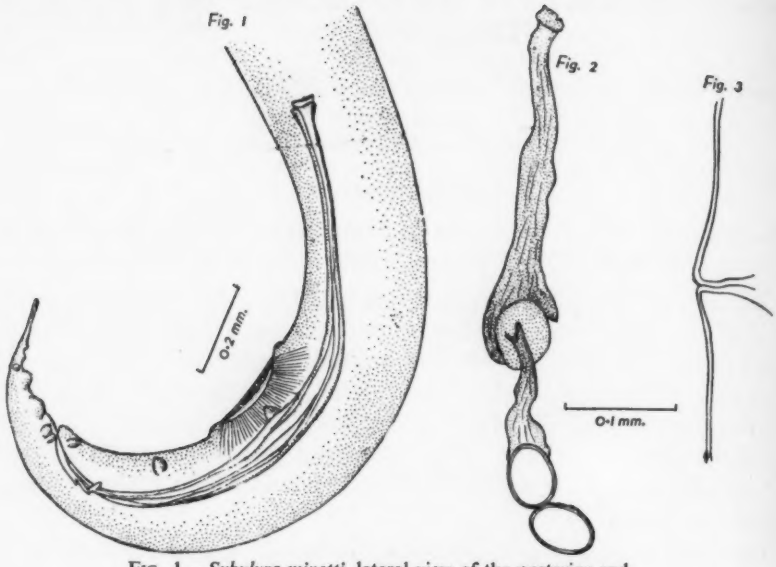

FIG. 1.—*Subulura minetti*, lateral view of the posterior end.
FIG. 2.—*Subulura minetti*, showing details of ovijector.
FIG. 3.—*Subulura minetti*, showing the opening of vulva.

by Cram (1927). The proximal, less muscular portion of the ovijector appears to open posteriorly into this hyaline area. The muscular sphincter described in some species was not noticed. The eggs measure 0·056–0·095 × 0·038–0·062 mm.

The species described above has some affinities with the species *Subulura strongylina* (Rudolphi, 1819) and *S. differens* (Sonsino, 1890), which are the only two species recorded so far from the fowl. *S. strongylina* has, however, eleven pairs of caudal papillæ. In respect of the number of caudal papillæ, the Indian species agrees with *S. differens* but it can be separated from the latter by the size of the spicules, the gubernaculum and the pre-anal sucker, by the vulva being situated on a prominence and by the character of the ovijector. These points of difference being quite distinctive, it is proposed to regard the Indian species as a new one for which the name *Subulura minetti* is proposed in honour of Dr. F. C. Minett, the Director of the Imperial Veterinary Research Institute, Mukteswar.

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DAMS AND FISHERIES; METTUR AND ITS LESSONS FOR INDIA

By B. Sunder Raj
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Power, irrigation, navigation and flood control dams are indispensable for the proper exploitation of a country's natural resources. Any artificial obstruction which prevents the free passage of migratory fish to and from their spawning ground is naturally detrimental to Fisheries, as it is bound materially to reduce, or even to prevent, the propagation and perpetuation of stock by the loss of upstream spawning and rearing grounds. To fishery administrators, the salmon and allied industries, and those interested in sport and the conservation of wild life, dams which unduly interfere with the normal life of fish have always caused grave concern or considerable apprehension. The adverse effects of dams on fisheries and their remedial measures have therefore engaged the serious attention of fishery officers, industrialists, sportsmen, biologists, and engineers for many years, especially from the middle of the nineteenth century.

Where dams were found indispensable, the accepted remedy till recently has been the construction of fish passes, ways, ladders, lifts and locks, which are devices to enable migratory fish to surmount dams or weirs. These are, however, expensive to build and to operate, but when suitably designed and properly worked have led to a satisfactory revival of fisheries destroyed by dams. Fisheries, being often an economically subordinate consideration, are apt to be ignored even in the advanced West, where their scientific and economic importance is fully realised and, a strong and well-informed public opinion presses for their recognition. In India the need to conserve all available water for drinking or irrigation, combined with the conditions in the rivers, which flow only during the rains or the season of melting snows on the Himalayas, renders fish passes relatively more difficult to build and to operate than in countries with perpetually flowing rivers and a super-abundance of water. Consequently two questions arise which require the most careful and thorough consideration ; first, to what extent dams prejudice fisheries in India; and secondly, where dams are shown to be injurious, what remedies are the most economical to adopt.

Historical

It is perhaps not generally known that the earliest fishery investigation in India, excepting those connected with the historic Pearl Fisheries, were inspired by a fear of possible damage to fisheries by dams or weirs. Dams have existed in Indian rivers from time immemorial, but they had mostly fallen into disrepair before the British occupation. It was not until the nineteenth century that the most important of them across the principal rivers in India were constructed or renovated. The middle of the nineteenth century witnessed a great revival of interest in salmon passes in England and America. Though primitive types of fish passes may be traced for several years previously, the most important types of fish passes were invented during this period. Among the early inventors of distinctive types the names of Smith, Cail and Malloch in Great Britain, and Brackett, Foster, Brewer and MacDonald in America, are well known. In response to a wide-spread demand for legislation, the Salmon Fisheries Acts of 1862 and 1868 in Scotland, and 1871 in England, were passed. Frank Buckland, a great champion of fish passes, during his term of office as Inspector of Salmon Fisheries in England, from 1866–88, designed and constructed several passes in England. Though the principles he enunciated have survived, not a single pass constructed by him remains.[1] In the meantime, in response to several appeals, the U.S.A. Commission of Fisheries was set up in 1871. Among the urgent fishery problems to which it devoted attention was the provision of fish passes.[2]

Actuated no doubt by the clamant public opinion at that time in the West, Sir Arthur Cotton, to whose skill and vision we owe many of the great irrigation systems, was the first to call the attention of Government in 1867 to the possible damage to Indian fisheries which might accrue from the seven weirs then completed. Dr. Day, who was at that time working on fishes in India, was immediately commissioned to investigate the question, and was subsequently appointed Inspector-General of Fisheries[3] in India. Thus it happened that simultaneously with the West, fish passes received attention in India. With characteristic energy and enthusiasm Day not only condemned dams as barriers to the passage of fishes and therefore highly detrimental to fisheries[4] but actually set himself to design a pass—a modified form of undersluice—which was tried at the Lower Anicut on the Coleroon River in South

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[1] T. E. Pryce-Tennatt, *Fish Passes*, 1938, 6.

[2] "U. S. Comm. of Fish and Fisheries," *Comm. Report*, 1872–73.

[3] F. Day, *Rep. on the F. Water Fish and Fisheries of India and Burma* (Calcutta, 1873), 1; also *Fishes of India*, 1878, Pref. p. 1.

[4] F. Day, *Ibid.*, 7–14.

India, and a model of which was placed in the Buckland Museum in 1869.[5] Hilsa, however, for which his pass was primarily designed, could not be induced to ascend it. Since Day's first pass, now in disrepair, so far as I can trace some seventeen other passes have been constructed in India; eleven in the Punjab,[6] two or three in the United Provinces[7] and one in Orissa.[8] The cost of construction of the more recent passes in the Punjab, to which province belongs the credit of having done the most in this direction, is estimated at over 6½ lakhs of rupees, besides the annual cost of repairs and the loss of water to agriculture. According to Southwell, the initial observations for a year in 1916 did not justify the fish pass at Cuttack (Orissa), while Edye did not consider the ladder at Haridwar (U.P.) effective. Hora and Mukherji[9] quote the secretary of the Dehra Dun Fishery Association's statement, that "it is true 'fish ladders' have been provided at places like Bhimgoda and Majapar, where the Northern Ganges canals take off but no one believes that fish of any size can come up these ladders." Fuller reports exist about the ladders in the Punjab. In the latest,[10] dated 1940, Dr. Khan concludes that most of these also are ineffective.

If ladders in India have not justified the expenditure and effort involved, it need not surprise us. Even in Europe and America, the perfect pass does not yet exist and the good ones are still in a minority.[11] "The devising of a fish pass is fraught with uncertainty, because it is almost impossible to prophesy the behaviour of fish and quite impossible to anticipate the vagaries of water. The subject involves a working knowledge of hydraulics, and while hydraulic engineers conversant with the habits and requirements of fish are rarely to be found, the rules and assumptions of hydraulics themselves are apt to be disconcertingly upset when applied to the functioning of a fish pass. The subject is by no means within sight of finality."[12] The report on a symposium on "Dams and the problem of migratory fishes" of the American Society of Ichthyologists[13] recently held at the Stanford University also shows that only a beginning has been made in the study of the subject. To a casual

[5] F. Day, "Fish Culture," *Int. Fish Exhib. Lit., Lond.*, 1884, **2**, 95.

[6] Hamid Khan, "Fish Ladders in the Punjab," *Journ. Bom. Nat. Hist. Soc.*, 1940, **41**, No. 3.

[7] Edye, E. H. H., *Rep. on the Fisheries of the U. P.*, 1923, 14.

[8] *Dept. of Fisheries Bengal Bihar & Orissa, Bull. No.* 11, 1918, 4.

[9] Hora, S. L., and Mukerji, D. D., "Fishes of the Eastern Deons, U. P.," *Rec. Ind. Mus.*, **38**, Pt. II, 135.

[10] Hamid Khan, "Fish Ladders in the Punjab," *Journ. Bom. Nat. Hist. Soc.*, **41**, No. 3, 561.

[11] T. E. Pryce-Tennatt, *Fish Passes*, 1938, 38.

[12] *Ibid.*, 7.

[13] *Stanford Ichthyological Bull.*, Stanford Uni., California, May 1940, **1**, No. 6.

observer, the building of a fish pass may seem a straightforward engineering enterprise, requiring only the application of conventional fish way designs and practices, but to no one with an intimate knowledge of modern fish ladders and of Indian requirements does the task seem so simple. Research and experiment have not reached the stage when general principles governing all dams can be enunciated. For success the design and operation of a pass must be adapted to the fish and to the physical conditions of the dam for which it is intended. Recent experience in England and America makes this self-evident proposition all the more clear. For instance, in one and the same river—the Columbia—and for the same fish—the ' sockeye ' and the ' chinook ' salmon and the ' steelhead ' trout—while ladders were possible and successful across the Bonneville and Rock Island Dams in the lower river, they had to be abandoned as impracticable for the Grand Coulee (350 ft. high) and Shasta (560 ft. high) Dams.[14] Moreover it was found that for one reason or another the lessons learned at one place, *e.g.*, Bonneville, would not directly apply to Grand Coulee or Shasta. If the task is so complex even with reference to a single river and the same fish, about whose instincts and habits so much is known, where are we in India ?

Despite the fact that fish passes attracted early attention in India, there is no evidence of any intensive study of the problem in the light of modern research and experience, even in the Punjab with its many fish passes. Following Day, many have tacitly assumed that dams are causing heavy, and in places, irreparable damage to Indian fisheries by intercepting the free passage of fish. Beyond the general biological assumption that any undue interference with the normal life of an animal will effect it adversely, no actual proof has been adduced, except possibly for hilsa in S. India. The belief appears to be based on hearsey or the statements of interested but for the most part uncritical observers. When at Mettur on the Cauvery river work began on the largest dam in the world at the time of its construction (1925–34) and even now the third largest, as Director of Fisheries in Madras, I seized the opportunity to investigate its effect on the fisheries of the river and begin intensive studies on the problem.

The Mettur Dam and the River Cauvery

(Plates XVI—XVIII)

A large storage reservoir to be formed by damming the Cauvery at Mettur was decided on in 1910[15] when the Madras Fisheries Department was in its infancy. It was sanctioned by the Secretary of State in 1924. During

[14] " Dams and the Problems of Migratory Fishes," *Stanford Ichthyological Bulletin*, May 1940, 1, No. 6, 195, 196, 199–205.

[15] " Souvenir of the Inauguration of the Cauvery, Mettur Systems," August 1934.

the interval the Fisheries Department does not appear to have been consulted, and by then it was too late to modify the scheme to meet fishery requirements, if any. The unique opportunity of studying the fisheries before and after the formation of the dam, however, remained. A brief account of the Cauvery River and the Mettur Dam is necessary for a correct appreciation of the problems studied.

The sacred Cauvery, the largest river south of the Kistna, rises on the Brahmagiri hills on the Western Ghats, near Mercara (Coorg), at an elevation of 4,400 feet (12° 25′N, 75°34′E). After a course of approximately 475 miles in a more or less south-easterly direction it enters the Bay of Bengal on the seaboard of the Madras district of Tanjore. The course of the river in Coorg is tortuous, on a rocky bed, with high banks covered with tropical vegetation. On entering the Mysore Plateau, it passes through a narrow gorge, with a fall of 60–80 feet, called Chunchankatte Rapids. It then widens to an average width of 300–400 yards, till it receives the Kabani, whence it becomes a much wider stream. At Sivasamudram are the celebrated Falls where the river makes a precipitous descent of 320 feet and enters the Madras Presidency. The Sivasamudram Falls are a complete barrier to the ascent of Fish. This materially assisted the study that has been undertaken, as it limits the possible effects of the dam to a stretch of the river from the Mettur Dam to Sivasamudram which is under the care and control of the Madras Fisheries Department. From Sivasamudram to Hoginakal Falls and for some four miles below, the river runs through wild hilly country, often in inaccessible rocky gorges. Some 32 miles above Hoginakal at the boundary of the Mysore State Panandu Chakravani (12 whirlpools) where after a fall of 30 feet strong vortices are formed during floods in a succession of deep rocky pools; and below this Makatattu (the Goat's Leap), where two rocks from the opposite banks approach each other,[16] are of interest as barriers to fish.

At Hoginakal Falls the river used to descend 70 feet in a deep chasm in its rocky bed, where it throws up a column of perpetual spray, resembling smoke, hence the name " Hoginakal " (Smoking Rock). The height of these Falls has, however, been greatly reduced by the formation of the reservoir above the Mettur Dam, which reaches these Falls. Fish are now able to ascend the Falls. Some of the catla which I introduced into the reservoir have been caught above the Falls. Below Hoginakal the river leaves the

[16] I am indebted for descriptions of these places to Mr. Bhimachar, Fisheries Officer, Mysore State, Rao Bahadur K. G. Belliappah, Madras Forest Service, and Mr. P. Shankunni Menon, Asst. Inspector of Fisheries, Mettur.

Mysore Plateau, and the Mettur Dam is placed in the gorge where the river enters the plains. The bed of the river, however, continues to be mostly rocky for more than 25 miles below the Dam, to its confluence with the Bhavani. A further hundred miles below, at Srirangam, close to Trichinopoly, the river divides into two branches, the Coleroon in the North and the Cauvery in the south, which embrace the Tanjore Delta. From the bifurcation the river is systematically tapped at several points by a network of irrigation channels, most of which have existed from the days of the Chola Kings of the eleventh century. The main flow of the river is through the Coleroon, which carries the bulk of the floods to the sea, and consequently the most important hilsa fisheries of the Cauvery occur in this branch. To this we shall revert later.

The Cauvery is affected by both the North-East and South-West monsoons. In Coorg and Mysore its main supply is received from the South-West monsoon, the first freshets generally occurring about the middle of June. The flow decreases in August and by October the river sinks to its normal dry weather level, consisting of straggling streams and a succession of deep rocky pools. Below the Mettur Dam the river is chiefly affected by the North-East monsoon and the floods due to this monsoon usually occur in November. The Bhavani, the Amaravathi and the Noyal are the chief tributaries below the Dam. Of these, the first two in their upper reaches are subject also to the South-West monsoon rains. Thus the Cauvery below the Dam has floods fairly continuously from June to January except for a short interval of six weeks between the two monsoons, in September and October. While the South-West monsoon supply is fairly dependable, the North-East monsoon is irregular and frequently fails. The object of the Mettur Dam is to remedy the unsatisfactory supply of the North-East monsoon by storing the floods of the South-West monsoon and distributing them evenly throughout the irrigation season up to January. The peculiar situation of the Dam between the upper and lower reaches, subject to two different monsoons, has also facilitated the investigation of fishery problems.

Of all the rivers in India the Cauvery is perhaps the most used for irrigation. It has over a dozen dams in actual use and is consequently the most suitable for the study of the problem of Dams and Fisheries in India. The Mettur Dam, about 250 miles from the mouth of Cauvery, is the fifth now functioning from the mouth. The four below it are the Upper Anicut, the Grand Anicut,—both across the main river—and the Lower Anicut across the Coleroon, which is the chief branch of the Cauvery (*vide* Plate XVI) and the Cauvery Dam across the Cauvery proper.

The Mettur Dam, one of the largest masonry dams of the world, is 5,300 feet long, 214 feet high at the deepest portion of the river and 171 feet broad tapering to 20·5 feet at the top, contains 54·6 million cubic feet, or 3,640,000 tons of masonry, and cost Rs. 480 lakhs to build. The reservoir formed by the Dam is a lake of 60 sq. miles, impounding 95,660 million cubic feet of water at its maximum level, which is 796 feet above mean sea level. Unlike the three dams below, the crest of the Mettur Dam will never be submerged, even during the highest floods. The normal flood discharge is through the Ellis surplus (*vide* Plate XVIII 2) to the East of the Dam with a sill level of + 770 feet. In times of extraordinarily heavy floods an emergency discharge is provided over a saddle to the West of the Dam + 790 feet above sea level. The Ellis Surplus has 10 vents of 60 feet span with liftable iron shutters. The channel from the Ellis Surplus is 3½ miles long and meets the river some 2½ miles below the Dam. The average gradient of the channel is about 1 in 100.

The table below shows the number of times and the number of days that the lake surplussed for the last five years :—

Year	Dates		Tolal No. of days	Total days for the year
	From	To		
1934	11—7–34	18—7–34	7	
,,	21—7–34	1—9–34	12	19
1935	20—9–35	25—9–35	6	
,,	25–10–35	30–10–35	6	12
1936	10—7–36	19—7–36	10	
,,	22—7–36	31— 8–36	41	
,,	1—9–36	1–11–36	62	
,,	12–11–36	31–12–36	50	163
1937	1—1–37	10—1–37	10	
,,	9—6–37	13—6–37	5	
,,	27—7–37	7—9–37	43	
,,	12–10–37	31–10–37	20	
,,	5–11–37	26–12–37	51	129
1938	7—7–38	25–10–38	110	110
1939	28—8–39	9—9–39	13	
,,	21–10–39	31–12–39	72	85
1940	1—1–40	2—1–40	2	
,,	7—6–40	10—6–40	4	
,,	3—7–40	31–12–40	182	188

From this table it will be seen that the Ellis Surplus flows on an average for about 100 days in the year. Though the Dam is an insurmountable barrier to fish, I anticipated that this large surplus channel with its easy gradient would assist in the passage of fish, and accordingly the free ascent and descent of fish has been actually observed every year since the building of the Dam.

When the level falls below + 774 feet (namely 4 feet above the sill level) the shutters of the surplus are let down and supply for irrigation starts from the High Level Sluice, which has 8 vents ($10\frac{1}{2} \times 16$ sq. feet) with a sill level at + 720 feet. Normally the lake is not expected to fall below the + 735 feet level. Should at any time the level fall below + 730 feet the irrigation supply will be through the Low Level Sluice which has five vents (7ft. × 14 ft.) with a sill level at + 670 feet. The Low Level Sluice is not expected to be used except in very bad years which may occur once in ten years or so. Thus the High Level Sluice is designed to operate with a head of water ranging from 54 to 10 feet, though 15 feet is the lowest expected. Generally this pressure is excessive for fish to ascend or descend safely. A few large catfish have been occasionally swept down and picked up with severe injuries when the sluices are opened for the first time after a long closure. Possibly some smaller fish are also brought down accidentally but escape injury and pass unnoticed on account of their small size. There are no screens for these sluices to prevent the passage of fish, but the casualties have been so few and far between that the expense of erecting screens was not considered to be justified.

Like most modern dams, the Mettur Dam provides for the generation of electricity. Four pipes through the Dam for turbines operating under a maximum head of 150 feet have been installed for a maximum production of 49,000 h.p. There are three sets of two turbines each in operation now. The diameter of the turbines is 6 feet, but the space between the blades is only 2 inches. Thus only very small fish can pass through them. The intakes are screened by bars to prevent large fish and other obstacles from entering the turbines. The only experiments known with hydraulic turbines and fish are those conducted at the Bonneville Dam on the Columbia River (U.S.A.) which was completed four years after the Mettur Dam. The turbines installed there are, however, larger, 23 feet in diameter; slower, 75 revolutions per minute; and work at a lower pressure, maximum head 55 feet. Experience at Bonneville showed that with such turbines screens were not required for the intakes. To the great relief of all concerned, no dead or injured fish have ever been seen in the turbines at Mettur, in spite of their smaller size and greater pressure and speed. The Mettur turbines, however,

have a water-cooling system which consists of branching tubes, half an inch in diameter, taking off from the common turbine intake, which circulates cold water for cooling bearing and transformer oils. During one period of eighteen months, on three occasions one or two small fish were found jammed in these tubes. The fish were young Mystus and Mastacembelus, both bottom fish which habitually resort to crevices. The casualties were so few and occasional that remedial measures were not undertaken. Thus the experience at Mettur has definitely advanced our knowledge of the effects of turbines on fisheries. Apparently fish avoid them and no protection against the entry of fish is required for electric turbines in dams.

Biological Studies

The purely biological studies planned consisted of :—

1. A survey of the fishery resources of the river some miles above and below the Dam before and after its construction.

2. The determination of the exact seasons and occasions when mass migration of fish occurs.

3. An analysis of random samples to determine the kinds, sizes and conditions of fish which congregate at the Dam or its sluices and the reasons for the migration.

1. *Survey of Fishery Resources* (Plate XVIII).

The 24 species mentioned in Plate XVIII were found to be the most valuable food fish in the sections of the river surveyed.

The limits of their distribution prior to the building of the Dam are indicated in the accompanying map. While the majority occur throughout from below Erode to above Sivasamudram the survey disclosed the fact that waterfalls act as natural barriers to the rest. This had not been previously recorded. Catla, which is stocked annually in the Mettur Reservoir, since the formation of the reservoir and the reduction in the height of the Hoginakal Falls has been able to ascend the Falls and is now caught above Hoginakal. Four species, *Cirrhina cirrhosa*, *Mystus aor* and *Mystus seenghala*, and *Labeo fimbriatus* do not occur above Pannendu Chakravani Falls, while five, *Silonopangasius childreni*, *Pangasius pangasius*, one species of Labeo, one species of Barbus, and *Anguilla bengalensis* stop with the Sivasamudram Falls. Our knowledge of the habits and life history of these fishes is too inadequate to attempt any satisfactory explanation of their distribution or the exact manner in which the natural falls at Pannendu Chakravani and Sivasamudram check the dispersal of some species but not others. For instance, while *M. punctatus*

is found below and above the Sivasamudram Falls, the larger species *M. aor and M. seenghala* stop with Pannendu Chakravani. Again, while Silonopangasius and Pangasius stop with the Sivasamudram Falls, Wallagonia and Callichrous are found throughout. Similarly, while the common eel (Anguilla) does not occur above Sivasamudram Falls the spiny feel (Mastacembelus) occurs throughout. The difference in distribution between species of Labeo, Barbus and Cirrhina are no less mysterious.

On the accepted principles governing the geographical distribution of fish in rivers it is possible that Sivasamudram was not an insurmountable barrier to fish before the species now found above and below it became established. The continuation or extermination of species above the Falls after they assumed their present character will argue the existence or absence of the amenities of life required by the respective fish. Should however the Falls prove more ancient than the species of fish concerned, it is possible that the diffusion or penetration of the species to the upper reaches was prevented by the inaccessibility of the reaches above the Falls. In either case the Falls are acting as impediments to the distribution of certain fish, and thus the question whether waterfalls and consequently dams act as barriers to the distribution of fishes in India has been conclusively settled by this investigation.

The Mettur Dam was completed in 1934. For the last seven years no difference in the distribution of any of the 24 species mentioned has been noticed except catla, which as previously stated now ascends Hoginakal. It does not seem likely that any notable change in the distribution will occur in future for want of inter-communication, as more or less normal exchange between the sections intercepted by the Dam is maintained by the Ellis Surplus for 100 days every year.

2. and 3. *Mass Migration of Fish, their Kinds and Sizes.*—

Fish congregate at the Dam whenever water is let down and there seems to be no particular reason for their migration. A repeated analysis of random samples shows that all the fourteen species, except possibly murrel, the spiny eel and the true eel are attracted in large numbers by flowing water in the Ellis surplus as well as in the High and Low Level Sluice Channels. The kinds of fish that assemble and their relative abundance is the same throughout the year. Also fish of all sizes are found, adult fish however being very few. At times some have ripe eggs and flowing milt. It is therefore clear that these migrations are not for breeding so much as for dispersal of species. Otherwise the heterogeneous collection of young and adult fish at all times of the year cannot be accounted for. If as is assumed

the migration is for dispersal of species an insurmountable dam cannot seriously damage the fisheries so long as sufficient stocks of fish and their breeding grounds exist on either side of the dam. Indeed, the occurrence and dominance of species of Barbus, Labeo and Wallagonia above and below Sivasamudram Falls, which no fish can ascend, can only be explained on this basis. Thus the second important principle which has emerged from the study is that when a species is found both above and below a barrier in undiminished numbers and sizes, it is evidence that either mutual exchange occurs during floods, or, when the barrier is really insurmountable, the required facilities are found both above and below the barrier and the fish are not being adversely affected.

The adverse effects of the Mettur Dam and possible remedies.—

The Mettur Dam, however, was expected seriously to affect the river fisheries for reasons other than the insurmountability of the barrier it may create, and this has already happened.

These other causes are:—

1. The prevalence of lacustrine conditions in the river above the Dam.
2. The moderation of floods in the Cauvery .
3. The increased diversion of water to agriculture.

The permanent rise in the level of water, the submergence of rocks and banks and the accumulation of silt in the bed of the reservoir have already occasioned changes. Prawns on the sandy beds and weedy banks have totally disappeared. A gradual extinction of species which do not survive in stagnant waters and their replacement by fish adapted to such conditions of life is inevitable. Only future observations will show the extent of the changes which will supervene in the course of time. The moderation of floods has already adversely affected the hilsa fisheries lower down, by helping the formation of sand bars across the several mouths of the Cauvery.[17] Floods of the usual violence no longer reach the sea and attract breeding hilsa. The hilsa fisheries of the Coleroon in consequence have declined seriously since the formation of the Dam. The heavy floods and rapid currents in the river were responsible for deep scours in the bed. Fish of all kinds, particularly the larger varieties, shelter in the deep pools thereby formed during most of the year. Since the construction of the Dam these deep pools are gradually being filled with sand and silt and no longer provide shelter to breeding fish to the same extent. There has been a notable decline in the size of fish caught

[17] Sundar Raj, B., *Mad. Fish Dept. Ad. Report*, 1938–39, 3.

in the lower reaches of the Cauvery, even as far down as Tanjore. Lastly, the Dam is conserving an appreciable amount of water which was previously available in the river and is diverting it for irrigation, and any diminution in the volume of water necessarily decreases its fisheries. The whole length of the Cauvery and its tributaries in the Madras Presidency, except certain reaches in reserved forests constituted as sanctuaries or sections explicitly conserved as breeding reaches, is auctioned for its fisheries. The revenues obtained by annual sales are not necessarily an index to the actual value of the fishery. Taken over a number of years, however, they give a fair indication of the prosperity or decline of the fisheries to which they relate. The table below gives the total annual fish rents of the Department mainly from the Cauvery river for a period of eleven years.

Year	Total Receipts	Year	Total Receipts
	Rs.		Rs.
1928–29	113,351	1934–35	73,169
1929–30	104,509	1935–36	60,739
1930–31	123,776	1936–37	69,738
1931–32	101,426	1937–38	82,783
1932–33	102,367	1938–39	75,540
1933–34	91,299		

While the statement includes revenue from certain tanks the steady fall noticeable since 1934 when the Mettur Dam was completed is only from the Cauvery Fisheries, and must be attributed to a deterioration of the Cauvery Fisheries caused by the Dam. But the decline is not due to its intercepting the passage of fish but to the other causes explained above, for which there does not seem to be any remedy. Here there seems to be a vital and irreconcilable conflict between the interests of Fisheries and those of Agriculture, and so long as Fisheries are not of sufficient economic value the claims of agriculture or other more valuable uses to which water can be put must necessarily come first. The loss to fisheries can, however, be partially remedied by adopting where possible intensive cultural methods and by exploiting the resources of the reservoir. The Mettur fish farm has been designed towards this end. Even in Europe and America with their salmon and trout fisheries the same conflict occurs. For example, the reaches of the Columbia River in the U.S.A., above the Grande Coulee Dam have had to be

abandoned and artificial propagation of salmon and trout has been resorted to in order to remedy the loss.[18]

The protective measures which have been adopted at Mettur on my recommendation are:—

1. The total prohibition of fishing in the Surplus and Supply Channels where fish congregate and are liable to ruthless destruction.

2. The cultivation of suitable varieties of fish in a fish farm at Mettur for the annual stocking of the reservoir.

3. The proper exploitation of the fisheries of the reservoir.

Conclusion

A little reflection will show that Indian conditions have no parallel in Europe and America, where the problem of dams and fisheries has been studied. Though seasonal floods and drought occur in some of the rivers of the South-West in the United States of America we have no information of fish passes in such rivers as the Red River, the Sabine and the Trinity. Except the Indus, the Ganges and the Brahmaputra with their sources in the perennial snows of the Himalayas, the majority of Indian rivers cease to flow during the dry months.[19] The torrential rains confined to a few weeks of the two monsoons cause heavy floods, charged with silt. For the rest of the year even the largest rivers dwindle to trickling streams. In many the flow entirely ceases. In all water stagnates in deep pools in the scours on the beds where large breeding fish take shelter. Water is usually scarce everywhere in the tropics. The utmost economy has to be exercised in its use so as to make the most of what is available. The chances, therefore, of providing running water except during floods for fish passes, particularly of the larger kind required for hilsa, are very remote. Indian river fisheries also are not of sufficient economic value to supersede the demands of agriculture. Fish in Indian rivers are adapted to an annual succession of floods and droughts. The natural change of a flowing river into a chain of stagnant pools for most of the year is not conducive to long-range migrations, or to a free and continuous exchange between the different reaches. The carp and the catfish, which form the bulk of Indian river fish, are more or less sedentary by nature and breed, feed and live their lives within their limited range. A dam, therefore, is but one more obstruction to long-range migrations. The fish undertake only short journeys to breed or feed into the shallow tributaries or inundated banks during the floods. Young fish, however, travel further afield, whenever a flow occurs, and push their way into even the shallowest of water courses

[18] *Stanford Ichthyological Bull.*, 1, No. 6, 195.

[19] Day, *Rept. Fresh Water Fish and Fisheries of India and Burma*, 1873, 5–6, Paras VIII and IX.

and drains in their search for new waters, this being their natural mode of dispersal. Hence at dams it is the fry and the immature fish that preponderate. A few breeders from the neighbouring pools may also appear. The analysis of fish congregating at the Mettur Dam demonstrates what happens. Further proof that the fish that congregate at the Mettur Dam are all local breeders and are not long-range migrants was accidentally afforded by the following curious phenomenon. One day after floods fish had congregated as usual in great numbers at the Ellis Surplus and all pools in the 3½ mile stretch of that channel were crowded with fish of all sizes. Further ascent had been barred by the closing of the shutters. An unexpected cloud-burst that evening caused heavy local inundations and the channel below the Ellis Surplus as well as the river below became flooded with muddy water for a few hours. The next day there was no sign of congestion anywhere in the channel and all the assembled fish had dispersed, thus proving the correctness of the assumption that the fish are local migrants and have spread to the inundated banks and streams.

The only migratory fish of importance in Indian rivers are the mahseer among carp, the cock-up (*Lates calcarifer*), the goonch (*Bagarius bagarius*), the eel (*Anguilla* spp.) and the Indian shad or hilsa.

The mahseer are perhaps more migratory than other carp but even they are limited in their range compared with the truly migratory salmon and shad. From what is known of the habits of the mahseer and my own personal observations they are confined to rocky hill streams and are hardly ever seen at any great distance from the hills.[20] Thomas records their ascent for breeding into shallow hill streams when the monsoon floods occur.[21] During floods the dams as a rule are well submerged and those like Mettur which are never submerged have wide surplus weirs so that there is no obstruction to the ascent of any large breeding mahseer. At the larger dams only young mahseer are sparingly found. Dr. Khan also records finding only a few young mahseer at some of the ladders in the Punjab. The adult breeders reside in deep rocky pools amidst hills and do not undertake long-range migrations. So long as these sanctuaries are preserved the destruction of a few young descending below large irrigation dams cannot seriously impair the mahseer fisheries of the upper reaches. This however is my experience in South Indian rivers, especially the Cauvery, with its many dams. Possible exceptions are the temporary dams said to be used by villagers in certain Himalayan valleys for grinding corn[22] and somewhat similar obstructions built for fishing hill

[20] Thomas, H. S., " Rod in India," II Edition, 84.
[21] *Report on Pisciculture in S. Canara*, 11–12.
[22] Day, F., *Rep. Fr. Water Fishes and Fisheries of India and Burma*, 14, Para XXII.

streams everywhere. These interfere with the sanctuaries necessary for the protection of mahseer fisheries.

The next two fish are too rare to be of economic importance and being predaceous they are best eliminated by dams or weirs. The eels are more common but nowhere afford valuable fisheries. Further, during floods, when most dams are submerged and water flows over surplus weirs in others, sufficient facilities for the passage of eels exist. Very high falls, such as Sivasamudram, however, prevent the ascent of eels.

The hilsa is thus the only migratory fish which appears to need protection against dams. Here also the habits of these fish and the range of their migrations do not suggest that fish passes will prove the best or the most economical remedy. In the first place hilsa do not migrate to the sources of rivers as do salmon and trout.[23] Thus many dams at a distance from the sea do not affect their migration. For instance the Dumagudam, Sunkesala, Mettur and Kannambady Dams in the Madras Presidency, and apparently all the dams of the Punjab, are too far from the sea to affect the migration of this fish. Secondly, the hilsa seem incapable of negotiating the usual fish passes. Atkins[24] reporting on fish ways in the United States of America in 1874 states, "Salmon and alewives whose migrations extend to higher and smaller streams than those frequented by shad are found to take more readily to narrow passage ways than the latter." In his report therefore he excluded all reference to the shad as so little success had attended the efforts till that time to induce shad to enter fish ways. In devising fish ways for the American shad an entirely different scale had to be adopted. "The lack of sufficient volume of water is doubtless the prime reason why shad have never freely ascended any fish ways, except two remarkably wide ones constructed at Columbia on the Susquehanna River"[25] and more recently at Bonneville on the Columbia River.[26] Such passes even if practicable are uneconomical in Indian rivers with their great scarcity of water.

A few dams in the Madras Presidency, however, do obstruct hilsa runs and the problem therefore received closest attention in Madras. Day's pass at Lower Anicut on the Coleroon specially designed by him for hilsa failed as we have already seen. With the establishment of the Madras Fisheries Department in 1907 the hilsa problem was re-investigated in 1909 by H. C. Wilson.

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[23] Day, F., 35, para LI.

[24] Atkins, C. G., "On Fish Ways," *Ann. Rep. Comm. Fish and Fisheries, U.S.A.*, 1872–73, 592–94.

[25] *Ibid.*, 600.

[26] *Stanford Ichthyological Bull.*, May 1940, **1**, No. 6, 184.

[27] *The Geographical Journ. London*, Oct. 1940, **96**, No. 4. *Harnessing the Columbia River*, G. B. Barbour, 241.

Ladders as a remedy were abandoned by him since hilsa had not been exterminated as Day had anticipated forty years before, and since there was no prospect of the spent fish and their fry returning to the sea even if hilsa were made to ascend dams during floods. To compensate for the loss of upstream breeding grounds and the possible adverse effects, a hilsa hatchery on the model of shad hatcheries in America was established by him. When hilsa eggs were actually hatched by me for the first time in 1914, the whole problem was re-examined and the decision to abandon ladders was confirmed.[27] Since then the design of fish passes has been greatly improved and it is now possible to build satisfactory passes for the shad as at Bonneville, provided sufficient water is available. Also much progress has been made in our knowledge of the habits and life-history of hilsa.[28] The experiments at the Hatchery showed *inter alia* that eggs require clear water. Since hilsa runs occur in heavy floods when the water is muddy the possibility of hilsa eggs hatching below dams in silt-laden waters is excluded, but the discovery of very young stages and hilsa of all sizes below dams in the Cauvery, the Kistna and the Godavery[29] in certain years showed that hilsa were breeding notwithstanding the dams.

The water below the dams having proved unsuitably muddy, two possibilities remained. First, that some breeders ascend the dams when they are submerged in very high floods and the young hatched higher up with the parents descend within a few hours or days even while the high floods last. The eggs are laid in the evening and take but twelve to eighteen hours to hatch. As the high floods last for a few days on each occasion there is a real possibility that this happens. The fact that very few spent fish are ever

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[27] Sundara Raj, B., " On the Habits of the Hilsa (*Clupea ilisha*) and their artificial propagation in the Coleroon," *Abstract in the Journ. and Proc. Asiatic Soc. Bengal*, 1917, 8, No. 2.

[28] Sundara Raj, B., *Ann. Ad. Reps. Madras Fish Dept.*, 1930–31 to 38.

Hora, S. L., 1938.—A Preliminary Note on the Spawning Grounds and Bionomics of the so-called Indian Shad, *Hilsa ilisha* (Ham) in the River Ganges. *Rec. Ind. Mus.*, XL, pp. 147–158.

Hora, S. L. and Nair, K. K., 1940.—Further Observations on the Bionomics and Fishery of the Indian Shad, *Hilsa ilisha* (Ham.) in Bengal Waters. *Rec. Ind. Mus.* XLII, pp. 35–50.

Hora, S. L. and Nair, K. K., 1940.—The *Jatka* fish of Eastern Bengal and its significance in the Fishery of the so-called Indian Shad, *Hilsa ilisha* (Ham.). *Rec. Ind. Mus.*, XLII, pp. 553–565.

Nair, K. K., 1939.—On some ealy stages in the development of the so-called Indian Shad, *Hilsa ilisha* (Ham.). *Rec. Ind. Mus.*, XLI, pp. 409–418.

Prashad, B., Horo, S. L., and Nair, K. K., 1940.—Observations on the Seaward Migration of the so-called Indian Shad, *Hilsa ilisha* (Ham.). *Rec. Ind. Mus.*, XLII, pp. 529–552.

[29] *Ann. Ad. Rept. Madras Fish Dept.*, 1933–34, 41.

caught, and very few breeders are ever seen above the anicuts appears to confirm this conclusion. While fish about to breed are caught in large numbers at the anicuts, the fish apparently do not linger once the eggs are laid. As the visit to the upper reaches above the anicut is confined to exceptionally high floods when all fishing is precluded, they escape notice.

Secondly, that the hilsa runs in the Cauvery, Kistna and Godavery consist of fish which were not all born in the same rivers. An interesting fact ascertained during the years of hilsa investigation in South India is that shoals of breeding hilsa in exceptional years visit the smaller rivers, for instance the Pennar, in the Nellore District, and the Pennaiyar in the South Arcot District, which are not barred by high dams. Mr. Wilson first recorded the appearance of hilsa in the Pennar estuary in 1914 and made provision for a hilsa hatchery at the Ippur Fish Farm close by. The failure of hilsa to appear in subsequent years prevented operations, but in 1940 a hilsa fishery of some magnitude occurred in the Kandaleru close by when 1,600 hilsa were estimated to have been caught. This and other occasional catches in small rivers have established the fact that hilsa unlike the salmon do not necessarily return to the rivers of their nativity for breeding but spwan wherever facilities offer. Thus an intermixture of broods from different rivers freely occurs. The same has been observed in America. According to Meek,[30] " reasons have been given from a statistical study of the fisheries of America to show that shads do not necessarily return to the same river." Unfortunately, like some other shoaling fishes, hilsa in sufficient numbers and prime condition are available only when they swarm up the rivers for breeding. Their capture during the breeding season is therefore unavoidable. On the evidence of the hatchery experiments mere prohibition of fishing at the dams was found to be pointless as the fish could not successfully breed there. No fishing of any consequence is possible during very high floods when presumably the fish get their chance to breed up-stream. The only remedy for the hilsa problem is therefore successful artificial propagation. The discovery of young and adolescent hilsa in reaches of rivers below the dams and in tanks and sheltered waters connected with them has materially enhanced the chances of rearing hilsa in places like the Collair Lake, whence a free passage to the sea is available to them. Artificial propagation will materially augment the catches in the larger rivers as a free exchange of hilsa between the rivers has been proved by the investigation. Thus the main reasons for abandoning fish passes for hilsa still hold good, namely (1) There is not sufficient water for effective passes for hilsa, (2) They are uneconomical considering the value of the fishery. Though as we have seen, the Mettur Dam has caused a serious decline in the

[30] Meek, A., *Migrations of Fishes*, 101.

a Fisheries of the Coleroon, it was not in the manner predicted by Day. ugh fish passes of the type required for shad in the dams close to sea would augment hilsa fisheries, under Indian conditions they are racticable.

We are therefore led to conclude that in India fish passes are scarcely necessary and even where practicable are not economical. There is, ever, no doubt that dams seriously affect fisheries, as has been proved Mettur, otherwise than by obstructing the free passage of fish, and all edial measures possible by wise administration, legislation and culture uld be sought and practised if the baneful effects of dams on fisheries are e circumvented.

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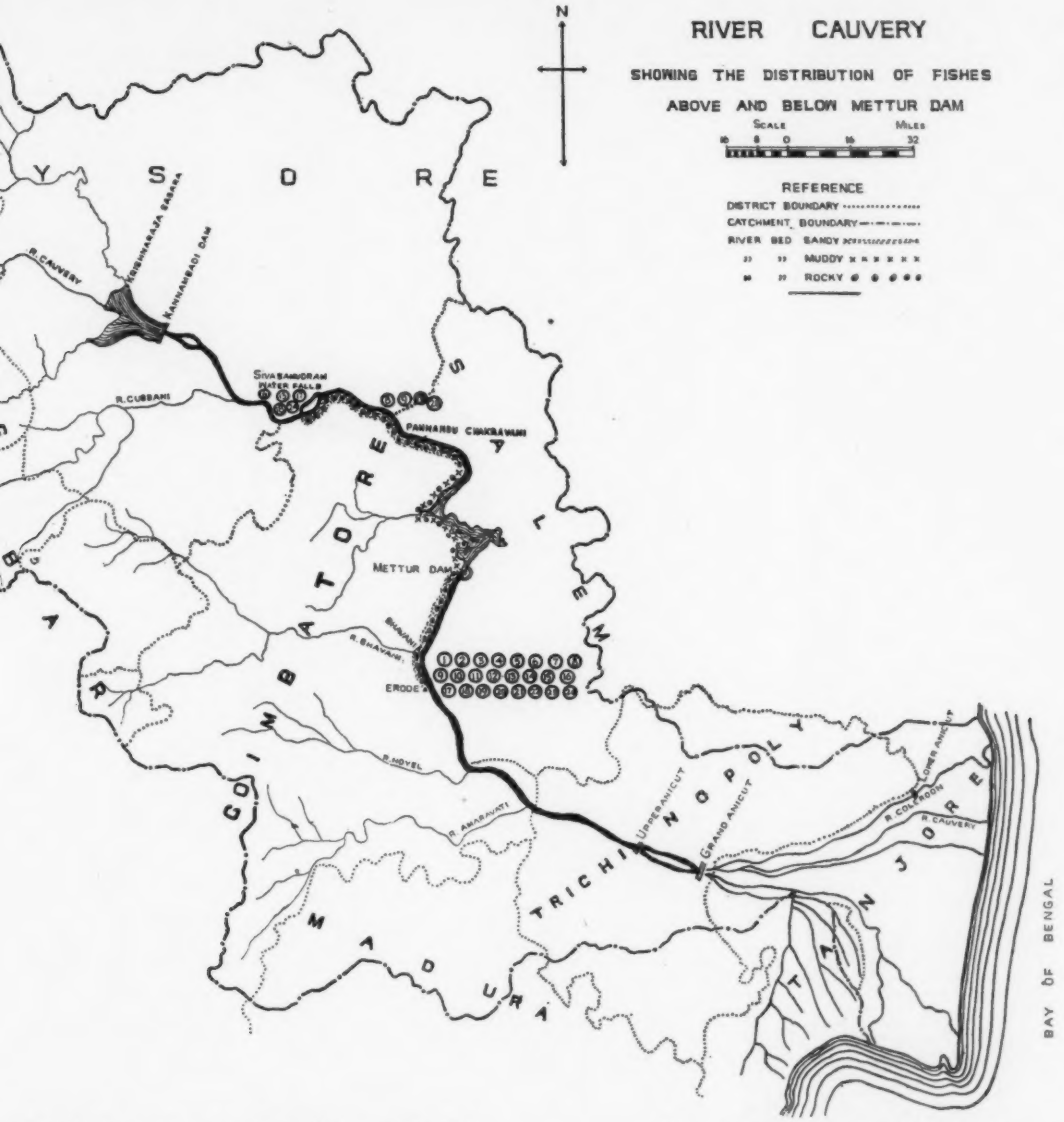
1. *Notopterus notopterus* (Pallas)
2. *Barbus carnaticus* Jerdon
3. *Barbus dubius* Day
4. *Barbus tor*
5. *Barbus sarana* (Ham)
6. *Barbus* sp.
7. *Catla catla* (Ham.)
8. *Cirrhina cirrhosa* (Bloch)



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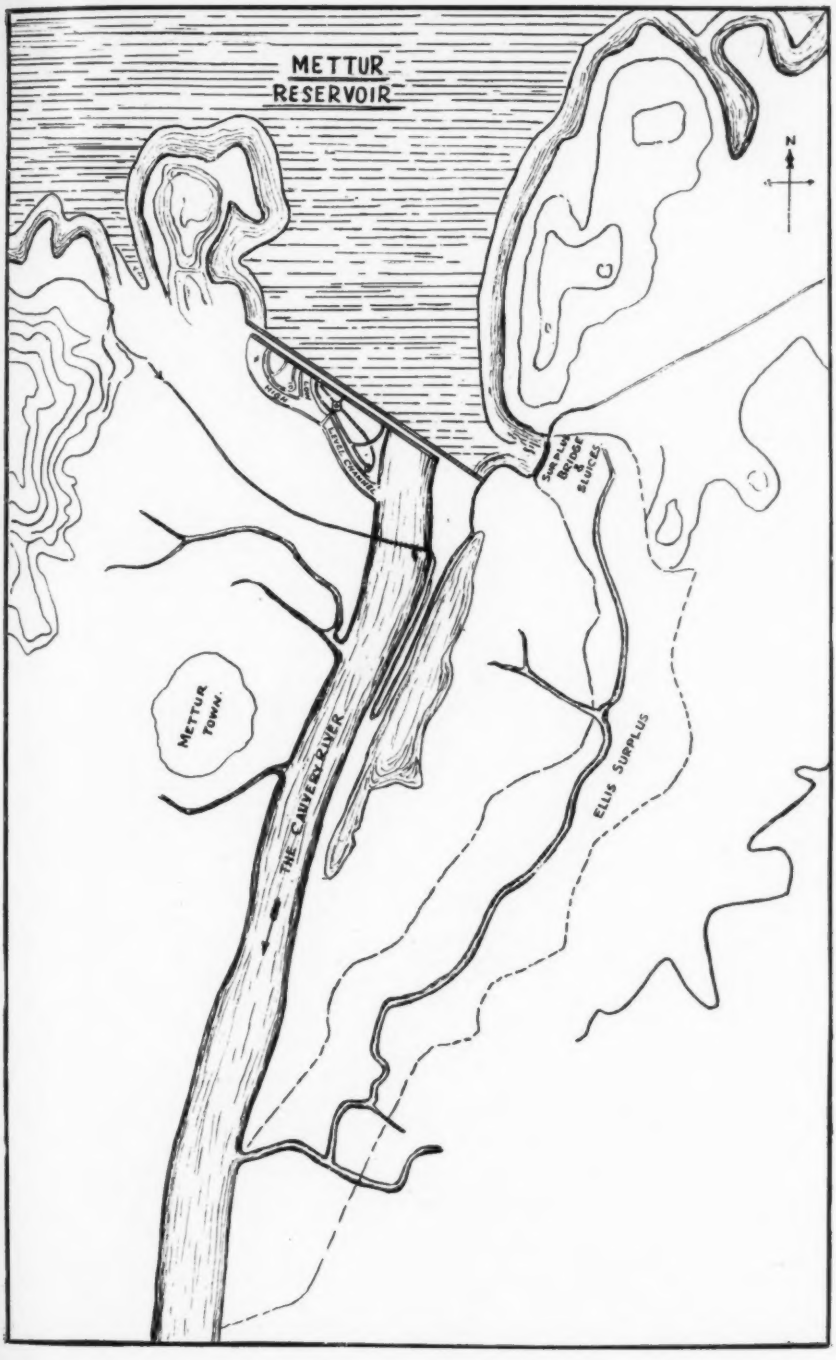
9. *Labeo fimbriatus* (Bloch)
10. *Labeo kontius* (Jerdon)
11. *Labeo calbasu* (Ham).
12. *Labeo ariza* (Buchanan)
13. *Labeo* sp.
14. *Scaphiodon brevidorsalis* (Day)
15. *Callchrous bimaculatus* (Bloch)
16. *Wallagonia attu Bloch* and Schneider
17. *Pangasius pangasius* (Ham)
18. *Silonopangasius childreni* (Sykes)
19. *Mystus aor* (Ham)
20. *Mystus seenghala* (Sykes)
21. *Mystus punctatus* (Jerdon)
22. *Ophiocephalus marulius* (Ham)
23. *Mastacembelus armatus* (Lacep)
24. *Anguilla bengalensis* (Ham. Buch.)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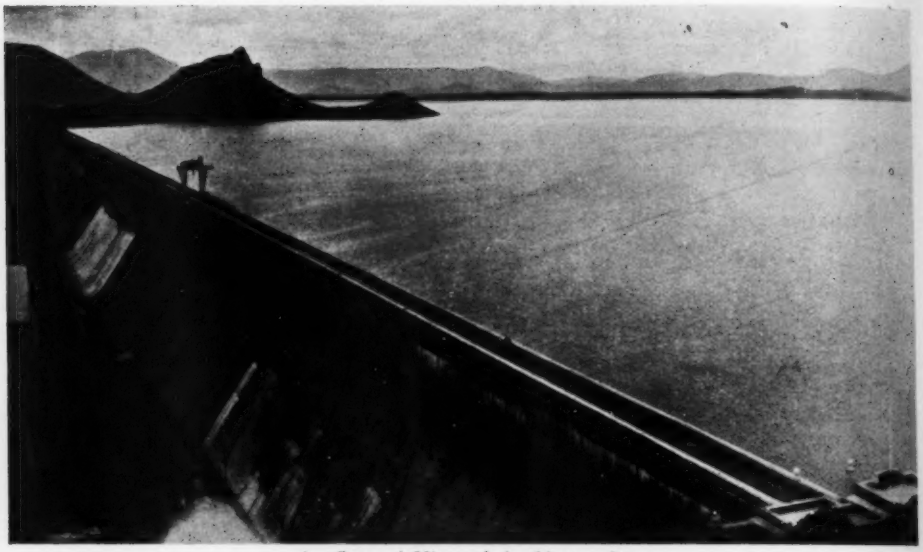
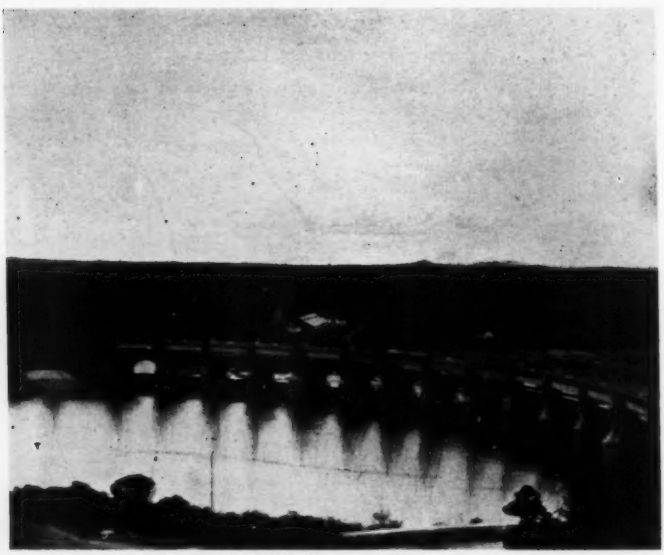


1. General View of the Mettur Dam



2. Ellis Surplus Sluices

THE GROWTH OF THE SUGARCANE PLANT IN INDIA

II. Physiological Effects of Deficiency or Excess of Added Fertilisers upon Growth Characters, Carbohydrate Metabolism, Yield, and Juice Quality of Sugarcane

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I. Introduction

Quality of cane juice and quantitative growth characters were observed in the course of the preceding paper of this series to be inseparably connected with the ability of the green leaf to synthesise food material and the relative efficiency of other related processes ; in this the part taken by nitrogenous, potassic, and phosphatic nutrients, applied singly or in mixtures, was considerable as judged by the metabolic responses in general. Each of the nutrients had a specific rôle to perform at a given dose. As the next step in the further analysis of the problem, it is the aim of the present contribution to study the physiological effects of the partial withholding or added supply of these nutrients upon growth characters, carbohydrate metabolism, yield and juice quality of cane at one or successive stages in its life-cycle.

Quantitative growth studies have revealed unquestionably that growth, development, yield, and composition of a plant are markedly conditioned by two sets of external variables, edaphic and climatic. While the latter is beyond the control of the cultivator, suitable variations in soil conditions, more particularly the nutritional level, have been found to greatly augment the output of matter and alter the composition of the plants. Upon this firm knowledge have developed the field technique and agronomic practices in crop production relative to manuring. In spite of considerable literature on the subject, the mechanism of response to increased or decreased supply of the nutrients is far from being clearly understood; there is scope for further analysis of the problem.

A number of investigators have made notable advances as to the effect of increased or decreased supply of fertilisers upon the growth and yield of the sugarcane plant. Thus Das[7] found that the greater the amount of nitrogen

conveyed by the roots, the more will be the requirement of carbohydrates, as a result of which comparatively small amounts of sugars would be stored by the plants. According to Hall,[14] as the amount of nitrogen is increased, the development of leaves and shoots increases and their maturity becomes more and more deferred.

Cross[5] has shown that application of phosphoric acid may induce early maturity in plots heavily fertilised with nitrogen. Varret[34] found that application of phosphorus has no effect on juice quality. Noel Deer[21] indicates that phosphorus has a beneficial effect on sugar content of cane, and potash a reverse effect. Varret[34] on the contrary has shown that application of potash has a definite effect on juice. Colen[4] found that a heavy dressing of nitrogen to soil poor in phosphoric acid and potash results in a greater foliage development without a corresponding increase in growth of cane. In order to obtain uniformly good results, balanced fertiliser mixtures have been recommended both from the point of view of safety and good results. Hach[15] has visualised that due to lack of potash too much nitrogen reduces the sugar content of the cane stalk. These generalisations are further supported by the observations of Sanyal[26] who found superphosphate to improve the quality of the juice, and sulphate of potash to maintain a high purity. Sulphate of ammonia gave the lowest sucrose and purity percentages and high percentage of glucose. Purity was also shown to be influenced by phosphoric acid treatments at Rio Pudras Experiment Station.[24] Hach[15] showed that due to ten per cent. of potash application the canes were so thick that they were unrecognisable. According to Moi[20] plots which gave no response to 100–150 lbs. of K_2O, gave an increase of 1½ tons of sugar for a 500 lbs. K_2O application. Lower levels of 100–150 lbs. gave even a decrease in yield while an increase in the amount of potash resulted in an increase in sugar, yield, juice quality, purity, etc. Vanden Honert[33] observed that decreasing nitrogen decreased the yield, height, number of shoots, etc. Phosphorus deficiency too caused a striking decrease in number of shoots; the cane plants became dwarf and leaves short and thick. Severe deficiency caused a stoppage of growth and decreased the length of the internode; potash deficiency had no effect on shoot formation.

The results of these and other workers, *viz.*, Taylor,[31] Cliff,[3] Dodds,[9] Kerr,[17] Turner,[32] and Sircar[30] have indicated beyond doubt that of the various nutritive ingredients determining the growth, yield, and juice characteristics of sugarcane, nitrogen, phosphorus, and potash are the main. The response of the cane to the various levels and ratios of these ingredients greatly varies from locality to locality and must greatly be determined by the soil and climatic conditions. To reduce soil heterogeneity the present series of

experiments were conducted in sand cultures grown in pots.* All possible combinations and levels of nitrogen, phosphorus and potash were applied for adjudging the extent and nature of response in yield and juice characters when either of these nutrients were supplied in excess or in deficiency.

II. Plan of the Experiments

Sugarcane *var.* Co. 312 used in previous experiments[27] was grown for purposes of detailed experimentation in standard size cement-concrete pots 18″ × 12″. In order to avoid leaching as also any exchange of ions between the cement concrete structure of the pots and the medium of growth, the pots were internally coated with paraffin-wax mixture. A mixture of clay-free coarse and fine sand in the ratio 1 : 3 was carefully washed, at first in running stream of water, and finlly treated with very dilute solution (about 5%) of caustic soda. The sand was well stirred and carefully washed for a number of days to remove the last traces of alkali. The pH of the sand was determined. Further washings were stopped when the washings were cleared of all sediments and the reaction of the medium reached a value in the vicinity of pH 7·0. Equally weighed quantities of sand thus washed was used for filling the pots.

Cane setts of uniform size containing one bud each were germinated in a sand bed separately. Soon after germination one sett containing a healthy, well germinated bud was planted per pot. When the first leaf was well developed, fertilisers were applied. Watering was done at intervals with measured volume of water and the water content of the medium maintained in the vicinity of 35% moisture-holding capacity; this was checked at intervals. To avoid leaching of nutrients, drainage from the pots was not allowed. Requisite quantities of sulphate of ammonia (20·6% N), superphosphate (40·2% P_2O_5), and sulphate of potash (48·0% K_2O) as determined by the various treatments were applied to each of the pots individually. The lay-out of the experiment is given below:—

SYSTEM OF REPLICATION : four randomised blocks of 43 treatment combinations.

TREATMENTS : Sulphate of ammonia (N); $N_1 = 18{\cdot}75$ lbs,; $N_2 = 37{\cdot}5$ lbs.; $N_3 = 75$ lbs. ; $N_4 = 150$ lbs. ; $N_5 = 300$ lbs.; $N_6 = 600$ lbs.; $N_7 = 1200$ lbs. of N per acre.

* The advantages of sand medium over water culture and the technique of the preparation of culture medium are given in another paper "The Growth of the Wheat Plant in India" I. (In course of publication.)

Superphosphate (P); $P_1 = 9{\cdot}37$ lbs.; $P_2 = 18{\cdot}75$ lbs.; $P_3 = 37{\cdot}5$ lbs.; $P_4 = 75$ lbs. $P_5 = 150$ lbs.; $P_6 = 300$ lbs.; $P_7 = 600$ lbs. of P_2O_5 per acre.

Sulphate of potash (K); $K_1 = 9{\cdot}37$ lbs.; $K_2 = 18{\cdot}75$ lbs.; $K_3 = 37{\cdot}5$ lbs.; $K_4 = 75$ lbs.; $K_5 = 150$ lbs.; $K_6 = 300$ lbs.; $K_7 = 600$ lbs. of K_2O per acre.

MEDIUM OF GROWTH : Well washed clay-free sand.

SIZE OF THE POT : $18'' \times 12''$.

DATE OF SOWING: March 22, 1938; March 27, 1939.

DATE OF HARVESTING: November 20, 1938; November 17, 1939.

TIME OF APPLICATION OF FERTILISERS: Soon after planting.

The quantity of fertiliser per pot was calculated on the top surface area basis of the pots. The rates corresponded approximately to field dressings on an acre basis. The details of the treatments are further given in Tables I–IV.

The percentages of sucrose, glucose, purity, °Brix, and juice were determined after the methods detailed in the preceding contribution of the series.[17] For the determination of carbohydrate fractions on dry weight basis of the plants, sampling was always done in the morning just before sunrise as recommended by Archbold.[1] A single bush was taken at random, the shoots severed off and the roots washed free from sand. The shoots and roots were carefully washed, cut into pieces and dried after the method of Link and Tottingham.[18] The dry weight of the tissues was determined after drying them finally in an air oven at 105° C.

Five grams of the dry material representative of the composite mass was taken in a flask containing about 150 c.c. of ether sulphuric and kept overnight so as to dissolve out the fatty substances of the cane tissue and thus facilitate the clarification of the sugar extract for analytical purposes. Next morning the ether was decanted off and the material washed twice with fresh ether to remove every trace of fat. The fat-free material was then extracted with 80% alcohol for six hours on a water-bath in a conical flask connected with a reflux condenser and filtered. The alcohol of the filtrate was removed by evaporation on a water-bath till only about 5 c.c. of the alcohol was left behind. Special care was taken not to allow complete drying of the alcohol and thus to save the sugars from possible charring. The residue after evaporation was taken up with water, made upto a known volume (200 c.c.). The solution thus obtained was clarified with solid lead-sub-acetate, filtered, deleaded with sodium biphosphate crystals and again filtered on a dry filter

paper, and the hexoses (reducing sugars) estimated after Munson Walker-Bertrand Method.[2,19]

For sucrose determination an aliquote amount (50 c.c.) of the above solution was taken in a flask, hydrolysed by heating with 5 c.c. of concentrated hydrochloric acid on a water-bath at 70° C. for 5 minutes,[2,19] cooling the flask quickly in running water just after the heating was completed. The acid was neutralised by adding a saturated solution of sodium carbonate till the liquid gave a slightly alkaline reaction. The entire solution was made upto 100 c.c. and clarified as mentioned above. Total sugars were determined in terms of reducing sugars, by Munson Walker and Bertrand's Method. One blank experiment was also carried out on the same lines as given above to determine the error, if any, in the estimations.

For the estimation of starch the plant residue after alcohol extraction was digested by the Taka diastase method.[2,19] The extract was decanted through the filter into a 250 c.c. volumetric flask, washing the residue thoroughly on the filter. 1–2 c.c. of the neutral lead acetate solution was added to the extract, the latter rotated, made to volume and mixed thoroughly. After filtering and deleading this extract, 200 c.c. of it was hydrolysed by refluxing for three hours with 100 c.c. of concentrated hydrochloric acid. The extract was then neutralised with sodium carbonate (solid) and its starch content determined in terms of reducing sugars by the same titration method as detailed in case of the glucose estimation.

For the determination of other characters such as yield, height, number of tillers, etc., the method followed previously[27] was adopted.

III. Experimental Results

A. Developmental Characters:

There is a close connection between the quantity of nitrogen supplied to cane and the growth characters: as the dose of nitrogen increases there results an increase in the height of sugarcane plants upto a level of 2 N, *i.e.*, 300 lbs. of N per acre; subsequent increases have little effect. Tillering, length of leaf, and girth of the stem too increase with increased application (Table I), optimum effect being attained for 150 lbs. N, 600 lbs. N, and 300 lbs. N per acre respectively.

Application of superphosphate has a slightly different effect: the height of cane, leaf-length, and girth reach the maximum limit at 75 lbs. P_2O_5; tillering is highest at 75 lbs. P_2O_5. Potash on the other hand, increases height and tillering upto 75 lbs. and leaf-length and girth upto 600 and 300 lbs. K_2O respectively.

TABLE I

Results of increasing and withholding supply of N, P and K upon growth characters of sugarcane at harvest

Characters	⅛ N	¼ N	½ N	N	2 N	4 N	8 N
Height ..	60·0	61·4	64·6	69·0	70·4	70·2	69·4
Tillers	2·0	2·0	3·0	5·0	3·0	3·0	2·0
Leaf length ..	40·0	41·8	42·0	43·0	48·0	49·0	45·2
Girth	2·0	2·0	2·0	2·5	2·9	2·5	2·5
	⅛ P	¼ P	½ P	P	2 P	4 P	8 P
Height	60·2	67·4	70·8	69·0	64·4	63·2	65·6
Tillers ..	3·0	4·0	3·0	5·0	3·0	3·0	3·0
Leaf length ..	41·0	45·2	47·8	43·0	43·1	42·8	44·0
Girth ..	2·0	1·9	3·0	2·5	2·6	2·0	2·0
	⅛ K	¼ K	½ K	K	2 K	4 K	8 K
Height of cane ..	50·0	54·2	62·6	69·0	63·6	62·0	60·6
Tillers ..	2·0	2·0	3·0	5·0	3·0	3·0	3·0
Leaf length ..	40·0	41·8	42·0	43·0	48·0	49·0	45·2
Girth ..	2·0	2·1	2·0	2·5	2·9	2·5	2·5
	⅛ (NP)	¼ (NP)	½ (NP)	NP	2 (NP)	4 (NP)	8 (NP)
Height	56·0	56·6	55·8	69·0	57·8	65·0	65·2
Leaf length ..	40·0	39·8	39·2	43·0	39·6	43·2	44·6
Tillers ..	3·0	3·0	3·0	5·0	4·0	4·0	3·0
Girth	1·8	2·0	2·5	2·5	2·0	2·5	2·5
	⅛ (NK)	¼ (NK)	½ (NK)	NK	2 (NK)	4 (NK)	8 (NK)
Height	45·6	49·8	49·8	69·0	53·0	57·2	51·4
Tillers ..	2·0	2·0	2·0	5·0	4·0	3·0	3·0
Leaf length ..	40·1	39·6	37·8	43·0	40·2	39·9	39·2
Girth ..	1·7	2·0	2·0	2·5	2·5	3·0	1·7
	⅛ (PK)	¼ (PK)	½ (PK)	PK	2 (PK)	4 (PK)	8 (PK)
Height ..	60·8	59·2	62·4	69·0	62·6	57·8	50·25
Tillers	3·0	3·0	3·0	5·0	4·0	4·0	3·0
Leaf length ..	40·8	40·9	41·2	43·0	42·2	40·1	39·3
Girth ..	2·0	2·0	2·0	2·5	2·7	2·0	1·7
	⅛ (NPK)	¼ (NPK)	½ (NPK)	NPK	2 (NPK)	4 (NPK)	8 (NPK)
Height	61·2	60·5	72·2	69·0	72·0	69·6	72·0
Tillers ..	2·0	2·0	4·0	5·0	5·0	4·0	3·0
Leaf length ..	40·2	40·0	47·0	43·0	45·0	41·0	39·0
Girth	1·5	2·0	2·0	2·5	2·5	2·0	2·0

Height, length of leaf and girth are indicated in inches.

Nitrogen when supplied in combination with phosphorus (potash as a basal dressing), increases height and tillering upto a level of 75 lbs. P_2O_5 and 150 lbs. N ; variations in length of leaf are not very marked in treatments

below the normal (NP) set where these ingredients were applied at 150 lbs. N and 75 lbs. P_2O_5 respectively. Maximum effect is attained at 8 NP and ½NP level in case of leaf length and girth respectively. With mixtures of nitrogen and potash (NK series), simultaneous increase of nitrogen and potash upto a level of 150 lbs. N and 75 lbs. K_2O favours height, tillering and leaf length; maximum girth is only attained at 600 lbs. N and 300 lbs. K_2O applied together. Application of phosphorus along with potash (PK series) produces optimal effects with respect to all the characters at 75 lbs. P_2O_5 and 75 lbs. K_2O added together.

When all the three fertilisers are supplied together (NPK series) increase in N, P and K simultaneously upto 75 lbs. N, 37·5 lbs. P_2O_5 and 37·5 lbs. K_2O respectively promotes height and leaf length; tillering and girth are maximum at 150 lbs. N, 75 lbs. K_2O and 75 lbs. P_2O_5.

It would thus appear that in series where two or more fertilisers are increasing simultaneously the optimum effect is generally obtained either at the normal dose* or below it; in case of single fertilisers (N, P or K series), the optimum effects are obtained at the normal dose or above it.

B. Assimilation, Respiration and Chlorophyll content:

In general, increase in nitrogen increases assimilation, respiration and chlorophyll-content of leaves; both assimilatory and respiratory activities are highest in plants fed with 150 lbs. N, while chlrophyll reached the uppermost limit at 75 lbs. N (Table II). Increase in dose of phosphorus also augments the assimilation and respiration rate and chlorophyll upto 75 lbs. P_2O_5 per acre; further increase in the dose resulted in no gain. With potash maximum effect is produced at 75 lbs. K_2O on assimilation and respiration, and at 37·5 lbs. K_2O on chlorophyll.

In cases when fertilisers were added in paired mixtures (two fertilisers series) simultaneous increases in nitrogen and phosphorus with a basal dressing of the remaining one, augment both assimilation and respiration upto 150 lbs. N and 75 lbs. P_2O_5 and produces maximum chlorophyll at 600 lbs. of N and 300 lbs. of P_2O_5 per acre. Mixtures of nitrogen and potash (NK) treated plants have highest assimilation and respiration at 150 lbs. N and 75 lbs. K_2O, and maximum chlorophyll at the highest dose of NK tried. In the PK series, assimilation and chlorophyll attain maximal values under 75 lbs. each of K_2O and P_2O_5, while respiration is maximum for the highest dose studied.

* The normal dose is one where N, P and K are present at 150 lbs. N, 75 lbs. P_2O_5 and 75 lbs. K_2O respectively.

TABLE II

Assimilation, chlorophyll content and respiration rates of mature leaves (age 28–29 weeks) 1938–39

Levels*	N	P	K	NP	NK	PK	NPK
			Assimilation				
1	6·7	7·4	78·7	6·3	6·0	8·31	10·70
2	3·3	8·3	·6	7·06	6·1	7·5	8·12
3	8·7	7·7	7·9	7·29	6·25	8·28	7·50
4	10·80	10·80	10·80	10·80	10·80	10·80	10·80
5	6·1	10·62	8·55	7·73	4·71	6·6	7·50
6	8·82	6·9	8·7	6·1	7·2	8·6	7·50
7	6·43	7·32	7·32	4·95	7·51	8·4	8·05
C.D. @ 1%	0·84	0·56	0·53	0·69	0·60	0·77	0·75
			Chlorophyll content				
1	10·6	5·9	15·5	9·6	14·7	9·6	6·5
2	11·0	5·6	15·5	12·5	16·5	8·0	7·4
3	18·9	5·7	20·5	18·0	14·8	9·1	6·4
4	18·6	18·6	18·6	18·6	18·6	18·6	18·6
5	15·2	13·2	13·2	14·8	18·7	11·2	13·0
6	14·9	14·0	12·0	19·0	15·5	9·9	14·3
7	17·7	10·8	8·7	17·6	21·2	13·4	8·6
			Respiration				
1	4·30	3·02	3·80	4·11	4·90	3·76	4·90
2	2·75	4·85	4·90	4·35	3·87	3·36	7·13
3	4·90	3·40	3·71	4·39	4·17	3·95	3·83
4	4·94	4·94	4·94	4·94	4·94	4·94	4·94
5	3·10	4·70	3·95	3·97	2·55	3·27	5·67
6	4·53	3·16	4·34	3·94	3·16	4·60	7·94
7	2·99	4·58	3·04	3·36	4·10	6·68	7·55

* Levels arranged in the same order as in Tables IV–IV (6).

Assimilation and respiration in mgm. of CO_2 per 100 sq. cm. per hour; chlorophyll in mg. per 10 gm. fresh weight.

In admixtures of all the three ingredients when all of them are simultaneously increasing, maximal chlorophyll and photosynthesis are attained at 150 lbs. N, and 75 lbs. each of P_2O_5 and K_2O; respiration is highest at 4 NPK treatment.

Considering all the series together it is noted that maximum assimilation, respiration and chlorophyll are attained at 150 lbs. N + 75 lbs. K_2O + 75 lbs. P_2O_5, 600 lbs. N + 300 lbs. P_2O_5 + 300 lbs. K_2O and 150 lbs. N + 75 lbs. K_2O and 75 lbs. P_2O_5 per acre respectively.

C. Carbohydrate Fractions in Sugarcane:

Varying the doses of nitrogen, phosphorus, or potash, induced a marked change in the carbohydrate fractions of the mature cane (Table III);

TABLE III

Carbohydrate fractions, on percentage dry matter basis of Co. 312
(*Sampling date 20th November* 1938)

Levels*	Reducing sugars	Sucrose	Starch	Total carbohydrates
		N Series (PK constant)		
1	7·5	16·2	25·4	48·4
2	8·1	24·3	20·1	54·3
3	6·8	24·0	17·2	47·1
4	9·5	24·0	30·5	54·9
5	5·5	17·2	29·4	52·5
6	5·5	17·3	30·3	52·9
7	10·5	14·6	24·1	48·3
		P series (NK constant)		
1	7·1	25·7	27·2	60·5
2	8·4	21·1	36·5	66·9
3	5·6	14·4	30·4	50·7
4	9·5	24·0	30·5	54·9
5	9·3	20·3	21·2	51·2
6	7·4	20·5	23·3	51·4
7	5·5	18·4	26·1	50·5
		K series (NP constant)		
1	5·5	11·9	20·5	37·4
2	8·4	14·4	23·4	47·80
3	8·2	13·7	27·3	49·2
4	9·5	24·0	30·5	54·9
5	7·5	15·8	29·6	53·6
6	10·7	24·7	31·6	67·0
7	6·3	16·1	28·4	51·1

* Levels arranged in increasing order as in Tables IV to IV (6).

when cane is fed with increasing doses of nitrogen with a basal dressing of 75 lbs. each of K_2O and P_2O_5 maximum percentage of reducing sugars is attained at the highest level of nitrogen applied; sucrose is highest at 37·5–150 lbs. N per acre ; starch and total carbohydrates are highest at 150 lbs.; further increase in the dose of nitrogen stands to no gain in any case.

Likewise, with nitrogen and potash as basal dresssings, increase in phosphorus affects the concentration of carbohydrates: highest percentage of reducing sugars is attained at 75 lbs. of P_2O_5, that for sucrose at 9·37 lbs. of P_2O_5, and those for starch and total sugars at 18·75 lbs. P_2O_5 per acre; increasing the dose beyond 75 lbs. P_2O_5 results in a steady decline in the percentage of all the carbohydrate fractions under study.

Increase in potash with a basal dressing of N and P increases the percentage of reducing sugars, sucrose, starch, and total carbohydrates all of which

attain the upper limit at 300 lbs. of K_2O per acre ; with smaller supplies each of the carbohydrate fractions appreciably decrease.

Clearly thus the supply of nitrogen, phosphorus or potash under basal dressings of the remaining two considerably alters the carbohydrate fractions of the cane, the relative magnitude of which is determined by the respective doses of the three fertilisers.

D. Juice Characters :

Sucrose.—With increase in the supply of nitrogen, sucrose percentage in the juice increases upto 150 lbs. N, further supply decreases it, the lowest sucrose corresponding with the highest dose tried [Table IV (1)]. There is not much to gain by increasing the supply of phosphorus [Table IV (2)]. Quite contrary to this, potash increases sucrose even upto 300 lbs. of K_2O per acre in both the years of experimentation [Table IV (3)]. Simultaneous increases in NP and NK do not favour sucrose formation; on the other hand it proves to be deleterious, although the harmful effect on sucrose is not as clear and characteristic as under the nitrogen series [Tables IV (4) and IV (5)]. With basal dressing of nitrogen, increases in phosphorus and potash simultaneously, however, increase the sucrose which is highest for 300 lbs. each of P_2O_5 and K_2O per acre [Table IV (6)]. Simultaneous increases in all the three ingredients has useful effects only upto the NPK level, subsequent increases tend to be unfavourable. Plants seem unable to take up the whole of the fertilisers added to pots; excess of the nutrients over the amount required by the cane results in depressing sucrose (Table IV).

Glucose.—Increase in nitrogen, in contrast to sucrose, increases the glucose percentage in sugarcane juice. Similar variations, though differing in degree only, are to be noted under increasing levels of P [Tables IV–IV (6)]. With potash, however, the differences between the minimum and the maximal doses are not so apparent: seemingly it has little to add to glucose. Simultaneous increases in NP and NK increase glucose; so is the case in the NPK series. Mixtures of phosphorus and potash, on the contrary, bring out little difference at the various levels.

°*Brix*.—The total solids increase with every additional dose of potash when applied with phosphorus, but decrease when the plants are fed with any combination of nitrogen and the dose increased (*i.e.*, NPK, N, NP, NK). In case of added phosphorus, however, increases upto 150 lbs. P_2O_5 favour a rise in the value of °Brix and subsequently climb down [Tables IV–IV (6)].

TABLE IV

Effects of simulataneous increases in N, P and K supply upon yield and juice quality of sugarcane (1938–39; 1939–40)

Treatments		Yield gms. per pot	Juice %	°Brix	Sucrose %	Purity coefficient	Glucose %
Levels	Symbols						
			NPK series				
$N_1P_1K_1$ ..	¼ (NPK)	605	61·3	15·2	12·8	78·9	0·8
		504	*56·6*	*15·2*	*12·75*	*83·8*	*0·7*
$N_2P_2K_2$ ..	½ (NPK)	802	62·7	15·4	12·2	79·2	1·0
		626	*61·0*	*15·4*	*13·0*	*84·4*	*0·5*
$N_3P_3K_3$ ..	¾ (NPK)	1380	63·2	15·6	12·6	80·7	0·9
		1352	*60·9*	*16·0*	*13·6*	*85·0*	*0·5*
$N_4P_4K_4$ ..	NPK	1426	63·5	16·4	13·6	82·9	0·6
		1473	*63·0*	*16·4*	*14·2*	*86·8*	*0·6*
$N_5P_5K_5$ ..	2 (NPK)	2419	64·7	14·4	12·0	83·3	1·8
		2154	*63·5*	*15·0*	*12·5*	*83·3*	*1·5*
$N_6P_6K_6$ ..	4 (NPK)	2921	65·2	13·4	6·7	50·0	2·2
		2991	*66·6*	*13·8*	*8·0*	*57·9*	*2·5*
$N_7P_7K_7$ ..	8 (NPK)	2377	60·8	12·4	5·0	40·7	3·0
		2123	*60·0*	*13·6*	*6·0*	*44·1*	*3·7*

Note.—Upper figures relate to 1938–39 and lower ones (italics) to 1939–40.

TABLE IV (1)

Results of increasing nitrogen supply (with basal dressings of P and K) upon yield and juice quality of sugarcane (1938–39; 1939–40)

Treatments		Yield gms. per pot	Juice %	°Brix	Sucrose %	Purity coefficient	Glucose %
Levels	Symbols						
			N series (PK constant)				
$N_1P_4K_4$ ..	¼ N	875	60·8	16·0	13·0	81·2	0·8
		898	*61·0*	*15·6*	*13·0*	*83·6*	*0·5*
$N_2P_4K_4$ ..	½ N	1088	65·3	16·2	13·2	81·4	0·9
		960	*62·8*	*16·0*	*13·6*	*85·0*	*0·6*
$N_3P_4K_4$ ..	¾ N	1023	66·4	16·2	12·9	79·6	1·1
		962	*64·0*	*16·6*	*14·0*	*84·3*	*0·5*
$N_4P_4K_4$	N		*Same as in NPK series*				
$N_5P_4K_4$ ..	2 N	1885	64·5	14·8	11·4	77·0	2·0
		1761	*63·6*	*15·0*	*12·5*	*83·3*	*1·6*
$N_6P_4K_4$ ..	4 N	2339	63·8	15·0	10·7	71·6	2·4
		2229	*59·8*	*15·0*	*10·5*	*70·3*	*2·6*
$N_7P_4K_4$ ..	8 N	399	62·2	10·2	3·7	36·7	3·3
		409	*60·7*	*10·0*	*3·2*	*32·5*	*3·5*

Note.—Upper figures relate to 1938–39 and lower ones (italics) to 1939–40.

TABLE IV (2)

Effects of increasing phosphorus supply (with basal dressings of N and K) upon yield and juice quality of sugarcane (1938–39; 1939–40)

Treatments		Yield gms. per pot	Juice %	°Brix	Sucrose %	Purity coefficient	Glucose %
Levels	Symbols						
		P series (NK constant)					
$P_1N_4K_4$ ..	¼ P	1398	63·6	15·4	11·4	74·0	0·8
		1490	*60·0*	*15·6*	*13·0*	*83·0*	*0·6*
$P_2N_4K_4$ ..	½ P	961	64·3	15·4	12·2	79·2	0·9
		881	*61·8*	*15·8*	*13·2*	*83·5*	*1·0*
$P_3N_4K_4$ ..	½ P	1307	64·5	16·4	12·9	78·6	0·9
		1279	*62·8*	*16·2*	*13·6*	*83·9*	*1·0*
$P_4N_4K_4$	P	*Same as in NPK series*					
$P_5N_4K_4$ ..	2 P	1369	62·7	16·6	12·7	76·5	0·9
		1293	*63·5*	*15·4*	*13·0*	*84·4*	*0·8*
$P_6N_4K_4$ ..	4 P	1782	62·8	15·6	12·2	78·2	1·2
		1711	*64·7*	*15·6*	*13·2*	*84·6*	*1·7*
$P_7N_4K_4$ ..	8 P	740	66·2	15·2	12·1	79·6	2·0
		798	*60·0*	*15·2*	*12·7*	*82·8*	*1·8*

Note.—Upper figures relate to 1938–39 and lower ones (italics) to 1939–40.

TABLE IV (3)

Results of varying potash supply (with basal dressing of N and K) upon yield and juice quality of sugarcane (1938–39; 1939–40)

Treatments		Yield gms. per pot	Juice %	°Brix	Sucrose %	Purity coefficient	Glucose %
Levels	Symbols						
		K series (NP constant)					
$K_1N_4P_4$ ..	¼ K	1574	63·5	16·6	13·8	83·4	1·0
		1538	*63·2*	*15·4*	*13·0*	*84·4*	*0·4*
$K_2N_4P_4$ ..	½ K	1086	60·6	16·2	13·5	83·3	0·8
		1117	*59·8*	*16·0*	*13·8*	*86·2*	*0·4*
$K_3N_4P_4$ ..	½ K	1240	62·5	16·4	13·5	82·3	1·2
		1319	*58·3*	*16·2*	*14·2*	*87·6*	*0·5*
$K_4N_4P_4$	K	*Same as in NPK series*					
$K_5N_4P_4$ ..	2 K	884	65·3	17·6	14·6	82·9	0·6
		966	*60·6*	*16·5*	*14·1*	*85·5*	*0·6*
$K_6N_4P_4$ ..	4 K	1019	62·7	17·6	15·4	87·5	0·8
		1074	*64·8*	*17·2*	*14·9*	*86·6*	*1·1*
$K_7N_4P_4$ ..	8 K	508	61·0	17·0	13·9	81·7	0·9
		508	*59·3*	*17·8*	*15·0*	*84·2*	*0·8*

Note.—Upper figures relate to 1938–39 and lower ones (italics) to 1939–40.

TABLE IV (4)

Effects of increases in nitrogen and phosphorus supply (with basal dressing of K) upon yield and juice quality of sugarcane (1938–39; 1939–40)

Treatments		Yield gms. per pot	Juice %	°Brix	Sucrose %	Purity coefficient	Glucose %
Levels	Symbols						
			NP series (K constant)				
$N_1P_1K_4$ ..	½ (NP)	781	60·6	17·4	14·2	81·8	0·5
		904	*60·6*	*15·9*	*13·6*	*85·5*	*0·4*
$N_3P_3K_4$ ..	¼ (NP)	947	61·7	17·0	13·7	80·8	0·5
		1062	*60·0*	*16·8*	*13·8*	*82·1*	*0·5*
$N_5P_5K_4$ ..	½ (NP)	1561	59·9	16·4	13·5	82·3	0·6
		1505	*57·8*	*16·1*	*13·9*	*86·3*	*0·6*
$N_6P_6K_4$	(NP)		*Same as in NPK series*				
$N_5P_5K_4$ ..	2 (NP)	2709	61·8	15·4	11·5	75·0	1·5
		2473	*62·5*	*15·2*	*12·6*	*82·8*	*1·4*
$N_6P_6K_4$ ..	4 (NP)	2380	63·6	14·0	9·3	66·4	2·4
		2307	*61·0*	*13·8*	*10·2*	*73·9*	*2·4*
$N_7P_7K_4$ ..	8 (NP)	2586	60·5	13·6	8·0	58·8	2·6
		2615	*58·8*	*14·0*	*8·5*	*60·7*	*2·3*

Note.—Upper figures relate to 1938–39 and lower ones (italics) to 1939–40.

TABLE IV (5)

Results of varying nitrogen and potash supply (with a basal dressing of P) upon yield and juice quality of sugarcane (1938–39; 1939–40)

Treatments		Yield gms. per pot	Juice %	°Brix	Sucrose %	Purity coefficient	Glucose %
Levels	Symbols						
			NK series (P constant)				
$N_1K_1P_4$ ..	½ (NK)	632	62·6	16·8	13·5	80·6	0·9
		584	*59·4*	*16·0*	*13·0*	*81·2*	*0·6*
$N_2K_2P_4$ ..	¼ (NK)	766	63·5	16·4	13·4	82·0	0·8
		789	*58·8*	*15·9*	*13·2*	*83·0*	*0·6*
$N_3K_3P_4$ ..	½ (NK)	1239	66·6	16·2	12·7	78·7	1·0
		1184	*59·0*	*16·5*	*13·1*	*83·6*	*0·3*
$N_4K_4P_4$ ..	(NK)		*Same as in NPK series*				
$N_5K_5P_4$ ..	2 (NK)	2291	64·3	16·4	12·6	76·8	1·0
		2095	*61·6*	*15·4*	*13·0*	*84·4*	*1·3*
$N_6K_6P_4$ ..	4 (NK)	2692	65·6	15·4	12·4	80·5	1·2
		2959	*62·5*	*15·2*	*12·4*	*82·2*	*1·3*
$N_7K_7P_4$ ..	8 (NK)	2163	63·2	15·4	11·4	74·0	1·9
		2169	*60·1*	*15·0*	*11·6*	*77·3*	*1·4*

Note.—Upper figures relate to 1938–39 and lower ones (italics) to 1939–40.

TABLE IV (6)

Effects of increasing phosphorus and potash supply (with basal dressing of N alone) upon yield and juice quality of sugarcane (1938–39; 1939–40)

Treatments		Yield gms. per pot	Juice %	°Brix	Sucrose %	Purity coefficient	Glucose %
Levels	Symbols						
			PK series (N constant)				
$P_1K_1N_4$ ..	$\frac{1}{8}$ (PK)	1631	62·6	16·0	13·3	83·5	0·9
		1598	*62·4*	*16·0*	*12·5*	*79·6*	*0·3*
$P_2K_2N_4$ ..	$\frac{1}{4}$ (PK)	2025	61·7	17·0	13·7	80·5	1·1
		1930	*61·8*	*16·4*	*13·5*	*82·4*	*0·6*
$P_3K_3N_4$ ..	$\frac{1}{2}$ (PK)	2191	60·8	16·4	13·1	79·8	1·0
		1999	*62·9*	*16·3*	*13·5*	*82·8*	*0·5*
$P_4K_4N_4$ ..	(PK)		*Same as in NPK constant*				
$P_5K_5N_4$ ..	2 (PK)	1996	63·2	16·6	13·6	82·2	0·6
		1850	*59·8*	*16·6*	*13·6*	*81·9*	*0·2*
$P_6K_6N_4$ ..	4 (PK)	2184	61·0	17·8	14·7	82·8	1·0
		2010	*60·7*	*17·6*	*15·1*	*85·7*	*0·8*
$P_7K_7N_4$ ..	8 (PK)	2019	60·5	16·6	13·3	80·4	0·8
		2001	*59·9*	*17·8*	*15·2*	*85·3*	*0·8*

Note.—Upper figures relate to 1938–39 and lower ones (italics) to 1939–40.

Relating to yield	1938–39	1939–40
S.E. per pot ..	±139·1	± 73·74
S.E. %	9·04	4·92
C.D. at 5% ..	194·73	101·1
C.D. at 1% ..	257·4	133·7

Purity.—The purity coefficient of the juice decreases with increasing levels of nitrogen when supplied singly or in combination with phosphorus or potash, *i.e.*, N, NP, NK and NPK. Addition of phosphorus or potash singly in increasing doses favours the maintenance of purity level, it being higher in the latter case; combined dose of phosphorus and potash produces similar results. Clearly thus nitrogen whether applied singly or in combination with either phosphorus or potash or both, lowers the purity of the juice [Tables IV–IV (6)].

Juice %.—The percentage of juice per unit fresh weight of the cane does not show marked variations from one treatment to another; nor are the effects of increasing levels of the fertilisers very promising. The percentage in all the series, in general, varies between 57 and 66 [Tables IV–IV (6)]. This is true for both the years of experimentation.

E. Yield of Stipped Canes :

Increases in nitrogen upto 600 lbs. with a basal dressing of PK, and in phosphorus upto 300 lbs. P_2O_5 with NK as a basal dressing, find favour in increasing the yield in both the years; increase in potash beyond the first level of 9·37 lbs. with NP as a basal dressing, has a depressing effect. Simultaneous increases in nitrogen and phosphorus upto 300 lbs. N and 150 lbs. P_2O_5 with K as a basal dressing, in nitrogen and potash upto 600 lbs. N and 300 lbs. K_2O with P as a usual dressing, and in phosphorus and potash upto 37·5 lbs. each of P_2O_5 and K_2O with N as basal dressing, produces maximum yield in both years in their own respective series [Tables IV–IV (6)]. When all the fertilisers are increased simultaneously, highest yield is obtained with 600 lbs. N and 300 lbs. each of P_2O_5 and K_2O per acre. Yield at this level is highest and differs significantly from all others.

Discussion

It has been noted in the previous pages that weight yield of sugarcane shows characteristic variations in response to different doses of nitrogen, phosphorus and potassium. When variations in yield are plotted against varying levels of N, P and K (Fig. 1) the values arrange themselves in a characteristic form; the curves derived indicate that with increase in the dose of nitrogen, an increase in yield is observed reaching a more or less optimal level at two and four times the normal dose of nitrogen. But corresponding increases in the levels of nitrogen application fail to cause proportionate increase in tonnage; a decline in yield follows. Raising the concentration of nitrogen beyond the optimum dose shifts the curves to a horizontal or declining position. This supports the contention of Das,[7] Das and Cornelison[8] that with higher applications of nitrogen beyond a certain level, yield of cane appreciably goes down due to intensive lodging induced by increased succulence. In Fig. 1 as many as four separate, yet essentially similar, curves are obtained in case of all the three fertilisers. The relative declination at which the curves are situated is determined to a large extent by the relative concentration of basal fertilisers other than the one under study. Thus the effect of nitrogen at a particular level is largely regulated by the level of phosphorus and potash associated with it. These observations are amply supported by the investigations of Desai,[6] and Sircar[30] and Singh *et al*[29] who have shown that each of the component nutritional ingredients has its own specific response the magnitude of which varies considerably in accordance with the nature of the interaction taking place between the amounts of nitrogen, phosphorus or potash supplied to the medium of growth.

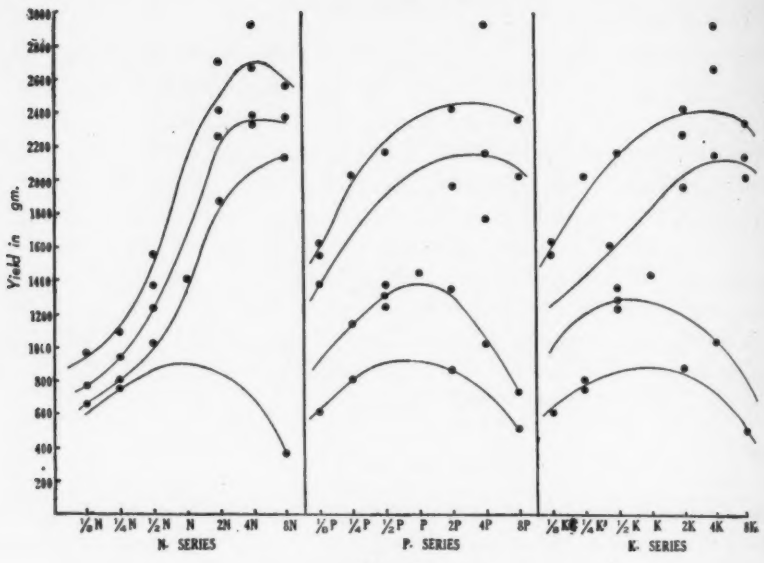

FIG. 1

Yield of stripped canes under varying levels N, P and K

The weight yield of cane also appears to have some specific relation with the quality of the juice (Fig. 2): (i) the higher the yield, the lower is the sucrose content, (ii) the higher the yield, the higher is the glucose content, and (iii) the lower the yield the higher is the purity of the juice. It is indicated therefore that yield of cane besides being dependent upon such external factors as irrigation, forms of manure, dose of nitrogen, and combination of N, P or K, is highly correlated with internal factors such as sucrose, glucose and purity. Not only that, the sucrose and glucose percentages amongst themselves also indicate a specific relationship when the values of any one are plotted against those of the other (Fig. 2). It is evident thus that higher the glucose percentage, the lower is the sucrose content and *vice versa*. Both yield and compositional characters are thus altered largely in response to the particular ratio and level of fertilisers.

The rate of assimilation also appears to be a smooth function of the dose of these fertilising ingredients (Fig. 3), but considerable variations in magnitude of assimilation are largely due to the presence or absence of nutrients other than the one under study. A slight variation in the dose of phosphorus or potash thus affects the intensity of assimilation under specific doses of nitrogen. The factor for association of different ingredients is therefore no

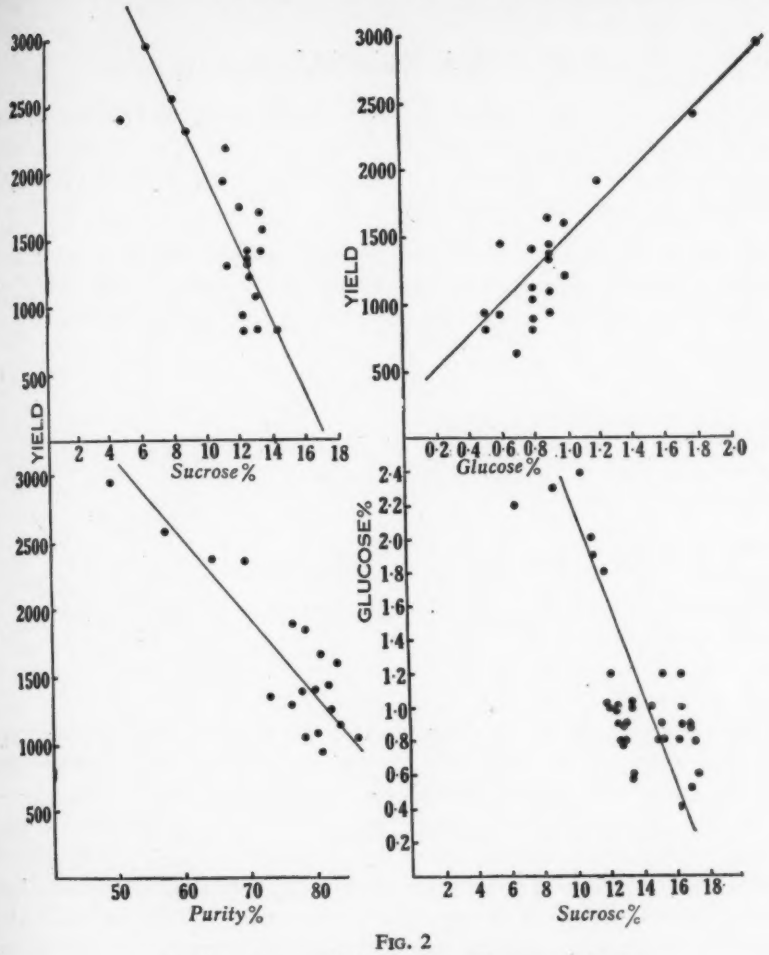

FIG. 2

Relation between Yield, Sucrose, Glucose and Purity

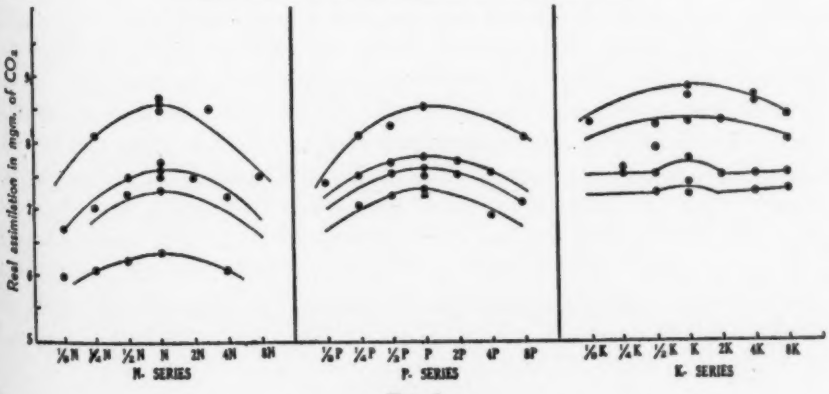

FIG. 3

Real assimilation as influenced by application of N, P and K

less important than the factors for age,[28] chlorophyll, and forms of fertilisers.[29] All the three ingredients, N, P and K, increase the photosynthetic efficiency of leaves, optimum effect being noticed at 150 lbs. N, 75 lbs. P_2O_5 and 75 lbs. K_2O per acre respectively.

So far as respiration is concerned nitrogenous fertilisers have altogether varying effects as compared to phosphatic and potassic ones (Fig. 4). Increase in nitrogen is usually associated with a decrease in respiration rate,

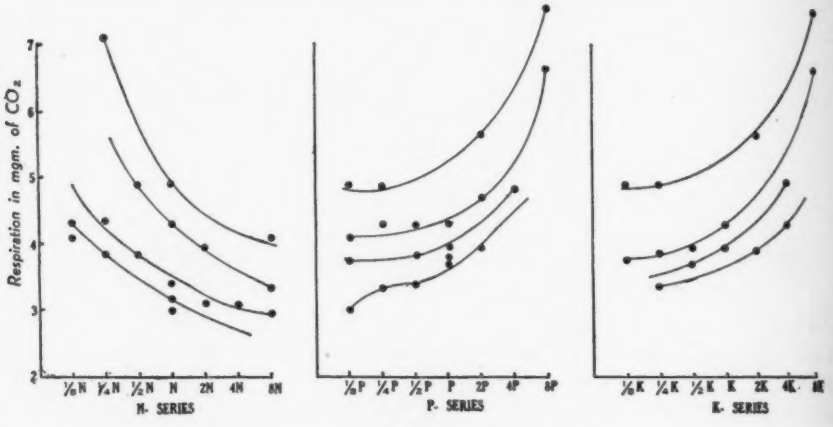

FIG. 4

Respiration as influenced by varying levels of N, P and K

whereas reverse is the case with phosphatic and potassic fertilisers. The considerable variations in the magnitude of respiration in different series also appear to be dependent upon the associations of different ingredients in consequence whereof the respiration curves shift their position considerably. Gregory and Sen[13] have shown that potash deficiency leads to much reduced sugars to normal proteins and to much increased amino acids in barley. On the other hand nitrogen deficiency is associated with increased sugar, much reduced proteins and amino nitrogen. It remains, however, to be seen whether high or low respiration rate is associated with variations in sugar and amino-nitrogen content of the tissues. Respiration and assimilation appear to be closely related processes: thus when the rate of respiration in the different series is plotted against the rate of photosynthesis (Fig. 5) it is shown that upto a certain limit the increase in the respiratory rate is definitely associated with increase in the photosynthetic activity but subsequently the rate of respiration falls.

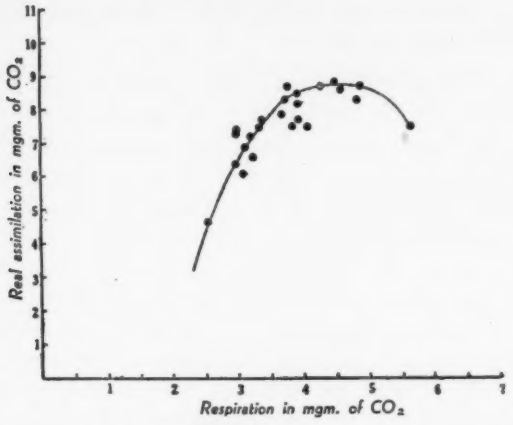

FIG. 5

Relation between assimilation and respiration rates of Sugarcane leaves

The chlorophyll content of the leaf also appears to be one of the several factors determining the intensity of assimilation under different nutritional conditions. While it is often observed that a high rate of assimilation under a particular combination of nutritive factors is associated with a high chlorophyll content and *vice versa*, there does not appear to exist any rigid relationship between the velocity of the photosynthetic reaction and the content of chlorophyll in leaves. When the chlorophyll content of leaves collected from plants grown in different fertiliser combinations is plotted against the respective values of photosynthesis, a characteristic curve is the outcome (Fig. 6). Upto a concentration of 15 mgm. of chlorophyll per 10 gm.

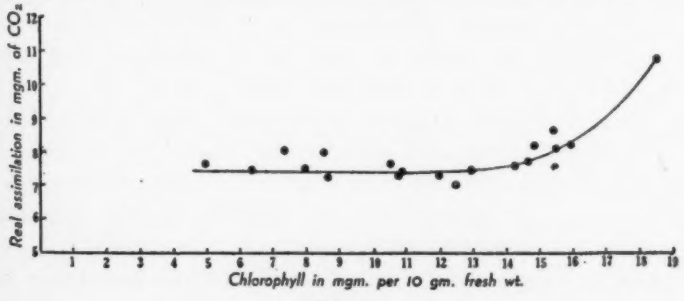

FIG. 6

Relationship between chlorophyll content and assimilation rate

fresh weight, increase in chlorophyll by the application of fertilisers does not appear to affect photosynthesis proportionately. Beyond this critical

concentration, however, high chlorophyll content appears to be associated with high photosynthetic activity.

The discussion of results give further prominence to the view held in the preceding section that the assimilatory and growth responses of plants to the nutrititonal conditions supplied is closely inter-connected and the analysis by no means simple. The effect is greatly influenced not only by the combination in which the manures are applied but also the concentration of specific ingredients supplied. The mechanism of response is all the more complicated in view of the fact that not only is the rate of assimilation affected, but variations are simulataneously induced in the chlorophyll content, in the compositional characteristics, and in various other directions as well. There appear to be many more factors which may as well be affected such as the uptake of salts, the rate of transpiration, the rate of translocation of food materials, by the application of fertilisers, emphasising thereby that the physiological analysis of fertiliser effect is much more intricate than what it is ordinarily understood and that for its thorough understanding, the response of all the different physiological activities to the presence or absence of nutritional ingredients needs to be investigated; this aspect will be stressed in a future communication.

From a practical point of view, however, the data lead to the generalisation that below and beyond a certain optimal range, certain characteristic changes take place in the growth behaviour of the plants. Taking the level NPK, where N was supplied at 150 lbs. N/ acre, P at 75 lbs. P_2O_5/ acre and K at 75 lbs. K_2O/ acre as the standard, the results of deficiency or excess of elements on the various growth characters are shown as under (Table V).

NITROGEN .. *Deficient supply* decreases height and girth of the stem; reduces starch and total carbohydrates in dry matter; diminishes the assimilation efficiency and chlorophyll content of leaf; yield is reduced.

Excess of nitrogen increases height and girth of stem; reduces the percentage of sucrose, purity and °Brix, and increases glucose percentage in juice; diminishes sucrose percentage in dry matter; lowers respiration and increases yield.

PHOSPHORUS .. *Deficient supplies of P* increase the percentage of starch and total carbohydrates in dry matter. Juice percentage is decreased; sucrose in dry matter is reduced.

Excess of P increases juice percentage and sucrose in dry matter; starch and total carbohydrates are reduced in dry matter.

TABLE V

Responses of N, P and K when present in excess or in deficiency, expressed as deviation from the standard fertiliser treatment

Characters	Nitrogen		Phosphorus		Potash	
	Deficiency	Excess	Deficiency	Excess	Deficiency	Excess
Height	− 7·0	+ 1·0	−2·9	− 4·6	− 13·4	− 3·6
Tillers	− 2·7	− 2·4	− 1·7	− 2·0	− 2·7	− 2·4
Leaf length	− 1·8	− 2·3	+ 1·6	+ 0·3	− 1·8	+ 4·4
Girth	− 0·5	+ 0·1	+ 0·2	+ 0·3	− 0·5	+ 0·1
Juice %	+ 1·8	− 0·8	− 0·45	+ 0·05	− 2·1	− 1·6
°Brix	− 0·3	− 3·1	− 0·6	− 0·8	− 0·2	+ 0·9
Sucrose	− 0·4	− 5·3	− 1·2	− 1·3	− 0·3	+ 0·7
Glucose	+ 0·11	+ 1·89	+ 1·09	+ 0·59	+ 0·87	+ 0·16
Purity	− 2·3	−22·9	− 4·5	− 3·8	− 0·3	− 0·1
Reducing sugars % dry matter	− 2·1	− 2·3	− 2·5	− 2·1	− 2·2	− 1·4
Sucrose % d.m. ..	− 2·5	− 7·7	− 3·6	+ 4·3	− 10·7	− 5·2
Starch % d.m. ..	− 9·6	− 2·6	+ 0·8	− 7·0	− 6·8	− 1·0
Total carbohydrates % d.m.	− 5·0	− 3·7	+ 4·4	− 3·9	− 10·1	+ 2·3
Real assimilation ..	− 4·6	− 3·7	− 3·4	− 2·6	− 2·8	− 2·7
Respiration	− 0·96	− 1·4	− 1·19	− 0·8	− 0·81	− 1·17
Chlorophyll	− 5·1	− 2·7	− 13·1	− 6·0	− 1·5	− 7·3
Yield	−481·9	+ 54·2	−230·2	−167·4	+1175·1	+ 203·5

POTASH .. *Deficiency of K* leads to reduction in length of leaf, girth of stem, °Brix, sucrose and total carbohydrates. Yield is increased.

Excess of K causes increase in length of leaf and girth of stem, °Brix, sucrose and total carbohydrates.

Mention in this connection may, however, be made of the researches of Russel[25] who has shown that plants deficient in potash are characterised by greatly reduced assimilation rate and considerable increase in respiration. Under phosphorus deficiency vegetative growth is restricted while assimilation

remains unaffected. Protein synthesis is greatly reduced under nitrogen deficiency and vegetative growth curtailed. Gregory and Richards[12] have shown that under phosphorus deficiency respiration is not markedly affected; under nitrogen and potash a marked drop and rise respectively in the rate of respiration is observed. Manurial deficiency, according to these authors, affects the rate of assimilation to a lesser extent than that of respiration.

Briggs [*Proc. Roy. Soc.* (B), 1922, **94**, 22–55] noted that subnormal photosynthetic efficiency from deficiency of nutrient salts was either due to the fact that the growing parts of the plant cannot utilise the carbohydrates as rapidly as the leaves can synthesise them, and thus the leaves are packed up with carbohydrates thereby reducing the photosynthetic activity, or the deficiency of the nutrient salts acts directly on the photosynthetic mechanism and thereby reduces the rate of assimilation.

Gregory and Sen[13] found that respiration rate in barley leaf is greatly reduced in the nitrogen-deficient and greatly increased in the potash deficient series. Gregory[10] has also shown that barley increases its leaf area but not its assimilation rate with addition of nitrates whereas addition of phosphorus and potash increases both asssimilation and leaf area. James[16] observed that starch formation per unit leaf area showed a significant increase in response to K when given in the form of sulphates. Richards[22,23] has shown that as the level of potash concentration is lowered, respiration rate increases but there is an optimum concentration below which the rate again increases. Singh and Lal[28] showed that increase in potassium content of wheat and linseed leaves is closely associated with rise in the photosynthetic activity during the adolescent stage of the life-cycle. Gregory and Baptiste[11] found that there is no significant difference in the photosynthetic rate in both the full-manured and nitrogen deficient series.

It would thus appear that the nature of fertiliser affect on the various growth characters of plants depends to a large extent upon the specificity of the crop as well. The differences observed have therefore to be carefully analysed in terms of the species under investigation, the conditions of nutritional supply, the conditions under which the investigations were conducted and measurements taken, and the contributions made by the existing soil and climatic conditions upon the growth behaviour of the plants. Until this is done, the responses in the case of different crop plants to the action of the same nutritive ingredients are difficult to be explained due apparently to the complexities involved as a result of the influence of the factors enumerated above. To this aspect of the question it is the intention to direct attention in a subsequent contribution of this series.

Summary and Conclusions

The effect is studied of nitrogen, phosphorus and potassium applied in the form of ammonium sulphate, superphosphate and sulphate of potash upon growth characters, assimilation and respiration rates, chlorlphyll content, carbohydrate content, juice characters, and yield of sugarcane *var.* Co. 312. The canes were grown in sand medium in standard size pots and supplied with these nutrients in varying levels soon after germination of the sets.

The following conclusions are the outcome of these investigations :—

1. With increase in the levels of either nitrogen or phosphorus or potash under basal dressings of the other two, or even under conditions of simultaneous increases in the level of two or more ingredients, the weight yield of cane increases upto a certain optimal level and subsequently shows a decline; the optimum limit for each of these ingredients varies within wide limits, depending upon the association and doses of other manurial ingredients.

2. The yield also appears to have specific relation with the quality of the juice, the higher the yield, the lower is the sucrose content; the higher the yield, the higher is the glucose content, and the lower the yield, the higher is the purity of the juice. It is also seen that the higher the glucose percentage, the lower is the sucrose content.

3. The rate of assimilation is a smooth function of the dose of the fertilisers applied but considerable variations in the magnitude are largely due to the presence or absence of other elements, in other words, the association of different ingredients plays a vital part in regulating the assimilatory response of leaves.

4. Increase in nitrogen is usually associated with a decrease in respiration rate, whereas the reverse is the case with phosphatic and potassic fertilisers. Upto a certain limit, increase in the respiration rate increases the photosynthetic activity, beyond which no proportionality in increase is obtained.

5. Upto a concentration of 15 mgm. of chlorophyll per 10 gms. fresh weight, increase in chlorophyll by the application of fertilisers does not appear to affect photosynthesis markedly. Beyond this critical concentration, however, high chlorophyll content appears to be associated with high photosynthetic activity.

6. The deficiency or excess of a particular ingredient brings about changes in the growth behaviour and compositonal characters of the plants. The responses noted in case of N, P or K series only are given below:—

NITROGEN.. *Deficiency of N* decreases height and girth of the stem, reduces starch and total carbohydrates in dry matter; diminishes assimilation efficiency and chlorophyll content of leaf; yield is decreased.

Excess of N increases height and girth of the stem, reduces the percentage of sucrose, purity and °Brix and increases glucose percentage in juice, diminishes sucrose percentage in dry matter; lowers respiration and increases yield.

PHOSPHORUS.. *Deficient supplies of P* increase the percentage of starch and total carbohydrates in dry matter. Juice percentage is decreased; sucrose in dry matter is reduced.

Excess of P increases juice percentage and sucrose in dry matter; starch and total carbohydrates are reduced in dry matter.

POTASH .. *Deficiency of K* leads to reduction in length of leaf, girth, °Brix, sucrose and total carbohydrates. Yield is increased.

Excess of K causes increase in length of leaf and girth; increase in °Brix, sucrose and total carbohydrates are noted.

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OBSERVATIONS ON THE PRODUCTION OF SEXUAL ORGANS IN PAIRED CULTURES OF PHYTOPHTHORA SPECIES OF THE PALMIVORA GROUP

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Introductory

During the course of the investigation of areca fruit rot caused by *Phytophthora arecae* (Coleman) Pethyb. an attempt was made to collect the well-known strains of *Phytophthora palmivora* from South India and Ceylon. The following are some of the strains collected and studied in this connection:

(1) *Phytophthora arecae* (Coleman) Pethyb. from *Areca catechu.*

(2) *P. palmivora* Butl. from *Borassus flabellifer.*

(3) *P. palmivora* Butl. from *Cocos nucifera.*

(4) *P. palmivora* Butl. from *Citrus nobilis.*

(5) *P. faberi* Maubl. from *Theobroma cacao.*

(6) *P. meadii* McRae from *Hevea brasiliensis.*

All the cultures except *P. faberi* were isolated from materials obtained from the west coast districts of the Madras Presidency. *P. faberi* was isolated from diseased cocoa fruits kindly sent by the Mycologist of Ceylon. These isolates were utilised for studying oospore formation in paired cultures.

Review of Previous Work

Kheshwalla (1935) has referred to previous literature on the subject from the time of Clinton (1909–10) who was the first to attempt mixed cultures for the production of oospores. Venkatarayan's work (1932) is not however mentioned.

As the present investigation deals only with mixed cultures belonging to Gadd's " rubber " and " cacao " groups and Ashby's " arecae " and " meadii" groups, it is necessary to review the relevant literature in order to understand the results presented here. Even though Clinton (1909–10) and Ashby (1922) have shown that oospores are produced in mixed cultures, Gadd (1924) was the first to undertake a systematic study of the subject specially confining

himself to Phytophthora strains belonging to the Palmivora (*P. faberi*) type and classified the strains he was dealing with into two groups, the " Cacao " group and the "Rubber" group. In a subsequent paper (1927), dealing with *P. arecae*, he found his isolation from areca palm not forming oospores with either of the two groups and therefore concluded that the areca strain is not a heterothallic strain of *P. faberi* but possibly, belongs to a species not allied to *P. faberi*. He also goes on to say that " the value of this negative evidence is doubtful as the result of growing an authentic strain of *P. arecae* with *P. faberi* is not known. Whether the former species would cross with *P. faberi* as does *P. parasitica* has not been determined".

Ashby (1929, 1) in a further comprehensive study of the strains belonging to the Palmivora group found that *P. palmivora* from coconut and palmyra palms in South India behaved like the members of the " Cacao " group forming oospores with the " Rubber " group.

Ashby (1929, 2) found *P. arecae* forming oospores with *P. meadii* and concluded that " both are one species separable by their behaviour in paired cultures into two groups of strains—the ' arecae ' and ' meadii ' groups comparable with the ' cacao ' and ' rubber ' groups of *P. palmivora*."

Narasimhan (1930) found *P. arecae* forming oospores in combination with Phytophthora species from *Santalum album* and *Jatropha curcas*. His isolate from *Loranthus longiflorus* also behaved like *P. arecae* in forming oospores with isolates from *Santalum album* and *Jatropha curcas*. He concluded therefore that Areca and Loranthus isolates are male strains and Santalum and Jatropha ones female strains. He also found *P. arecae* forming oospores with *P. meadii* and *P. parasitica*.

Venkatarayan (1932) compared his isolation of *P. arecae* from bud rot of areca palm with the usual strain from Mahali (fruit rot of areca palm) and found no difference in their behaviour in paired cultures with the isolate from *Sanatalum album*. He also found *P. arecae* forming oospores with a Phytophthora species resembling *P. palmivora* isolated from *Aleurites fordii*.

Material and Methods

Only six isolates of Phytophthora species mentioned earlier were used throughout the investigation for the study of oospore formation in paired cultures. The cultures were all grown either in petri-dishes or test-tubes. Three of the common media, Quaker oats agar, French bean agar and cornmeal agar were used. All the inoculated plates and tubes were incubated at 20° C. as there was no oospore formation at the laboratory temperature of 27–28° C. All the isolates were monosporangial in origin. The inocula were

planted about an inch apart in the test-tubes and about 1½ to 2 inches in the petri-dishes.

Results of Paired Culture Studies

Paired cultures in all possible combinations of the six isolates were tried in the three media mentioned earlier. Though oospores were produced in all the three media in such of those combinations that produce them, maize meal was found to produce them in larger numbers. The results obtained with the various combinations of paired cultures in maize meal agar are recorded in the tables given below. Table I gives the combinations which formed oospores and Table II those which did not form any oospores.

TABLE I

Measurements of 200 Oospores from the Central Zone of Mixed Cultures in Maize Meal Agar

Nature of isolates grown together	Oogonia			Oospores		
	Mean	Greatest frequency or mode	Range	Mean	Greatest frequency or mode	Range
1. *P. arecae* + *P. meadii* ..	35·7	35·0	28·00–42·00	31·9	31·5	24·50–38·50
2. *P. arecae* + *P. palmivora* from palmyra	33·6	31·5	28·00–40·25	29·8	28·0	24·50–38·50
3. *P. arecae* + *P. palmivora* from coconut	33·95	31·5	28·00–42·00	30·8	28·0	24·50–38·00
4. *P. arecae* + *P. palmivora* from citrus	29·4	31·5	24·50–33·25	27·3	29·75	22·75–31·50
5. *P. arecae* + *P. faberi* ..	28·0	28·0	18·25–33·25	24·5	24·5	17·50–29·75

TABLE II

Nature of isolates grown together	Oospore formation
1. *P. arecae* + *P. arecae*	..
2. *P. meadii* + *P. palmivora* (palmyra)	..
3. *P. meadii* + *P. palmivora* (coconut)	..
4. *P. meadii* + *P. palmivora* (citrus)	..
5. *P. meadii* + *P. faberi*	..
6. *P. faberi* + *P. palmivora* (palmyra)	..
7. *P. faberi* + *P. palmivora* (coconut)	..
8. *P. faberi* + *P. palmivora* (citrus)	..
9. *P. palmivora* (coconut) + *P. palmivora* (palmyra)	..
10. *P. palmivora* (coconut) + *P. palmivora* (citrus)	..
11. *P. palmivora* (palmyra) + *P. palmivora* (citrus)	..

Besides these, *P. meadii* was grown in combination with *P. parasitica* and was found to produce oospores in Quaker oats and French bean agar tubes respectively.

It will be seen from the results obtained that :

(1) *P. arecae* produces oospores when grown in combination with isolates of Phytophthora belonging to the " cacao " group and therefore behaves as a member of the " rubber " group.

(2) *P. meadii* behaves as one of the members of the " cacao " group.

(3) *P. palmivora* from palmyra and coconut palms, *P. palmivora* from citrus, *P. faberi* from cacao and *P. meadii* from *Hevea brasiliensis* belong to the "cacao" group and do not produce oospores when grown in combination with one another.

Discussion

Gadd (1924) was not able to get oospores when his *P. arecae* was grown in combination with either the " Cacao " or the " Rubber " group. When Ashby (1929, 2) took up the studies with *P. arecae* and *P. meadii* he confined himself only to the study of these two species evidently relying on the conclusions of Gadd as to the inability of *P. arecae* to produce oospores with either of the groups proposed by Gadd. Ashby even went so far as to suggest that both these are one species separable by their behaviour in paired culture into two groups of strains—the " Arecae " and " Meadii " groups comparable with the " Cacao " and " Rubber " groups. Narasimhan (1930) found *P. arecae* forming oospores with Phytophthora species from Santalum and from Jatropha. His studies however are of little value as most of his combinations with *P. arecae* were studied with unidentified species of Phytophthora with an implied suggestion that the strains he was dealing with were all strains of *P. arecae*. In describing the isolate from Jatropha however, he says that it belongs to *P. faberi* type. It is not possible to identify the Santalum and Loranthus isolates from the meagre data given. Relying on the absence of chlamydospores for these two strains he evidently thought that they should belong to *P. arecae* type. Venkatarayan (1932) found *P. arecae* forming oospores with the *Phytophthora* sp., from *Aleurites fordi* and also identified the latter as related to *P. palmivora* (*P. faberi*).

Narasimhan (1930) also obtained oospores when *P. arecae* was grown in combination with *P. parasitica* or *P. meadii* and concluded that *P. parasitica* behaved like a female strain. The present investigation has shown that *P. arecae* forms oospores with *P. faberi*. Thus according to Narasimhan's view *P. faberi* also should be a female strain. Lester-Smith (1927) has shown that *P. faberi* also forms oospores with *P. parasitica* and according to Narasimhan both these should be female strains. Besides, oospores have been obtained by the writer when *P. meadii* and *P. parasitica* are grown together. Thus it will be seen that Narasimhan's views are not tenable.

Uppal and Desai (1939) have shown that isolations of *P. arecae* from different localities when grown in different combinations produce oospores in some of the combinations, thus proving the existence of plus and minus strains of *P. arecae* in nature. The writer also isolated *P. arecae* from four different places and tried all possible combinations. None of them was found to produce oospores.

From the statements of the results presented here, it will be seen that *P. arecae* forms oospores with *P. palmivora* from palmyra and coconut palms and from *Citrus nobilis*, *P. faberi* and *P. meadii*. The areca strain therefore falls naturally in the rubber group of Gadd's classification. *P. meadii* behaves like *P. palmivora* and *P. faberi* and should therefore be placed in the "cacao" group. It will thus be seen that Ashby's second group is superfluous as *P. arecae* behaves like a member of the "rubber" group and *P. meadii* of the " cacao " group. The results given in this paper are the first definite record of the formation of oospores of *P. arecae* with known members of Gadd's " cacao " group. The relationship of *P. meadii* to the " cacao " group is also established for the first time.

Tucker (1931) considers that *P. palmivora*, *P. arecae*, *P. meadii* and *P. faberi* as strains of one and the same species, *viz.*, *P. palmivora*. The formation of oospores in combinations of *P. arecae* with *P. meadii* or any one of the members of the " cacao " group lends additional support for merging all these isolates as strains of *Phytophthora palmivora*.

I wish to express my indebtedness to Mr. K. M. Thomas, Government Mycologist, for help and advice during the course of the work and in the preparation of this paper.

Summary

Oospore formation in paired cultures with various combinations of six isolates of Phytophthora species were studied.

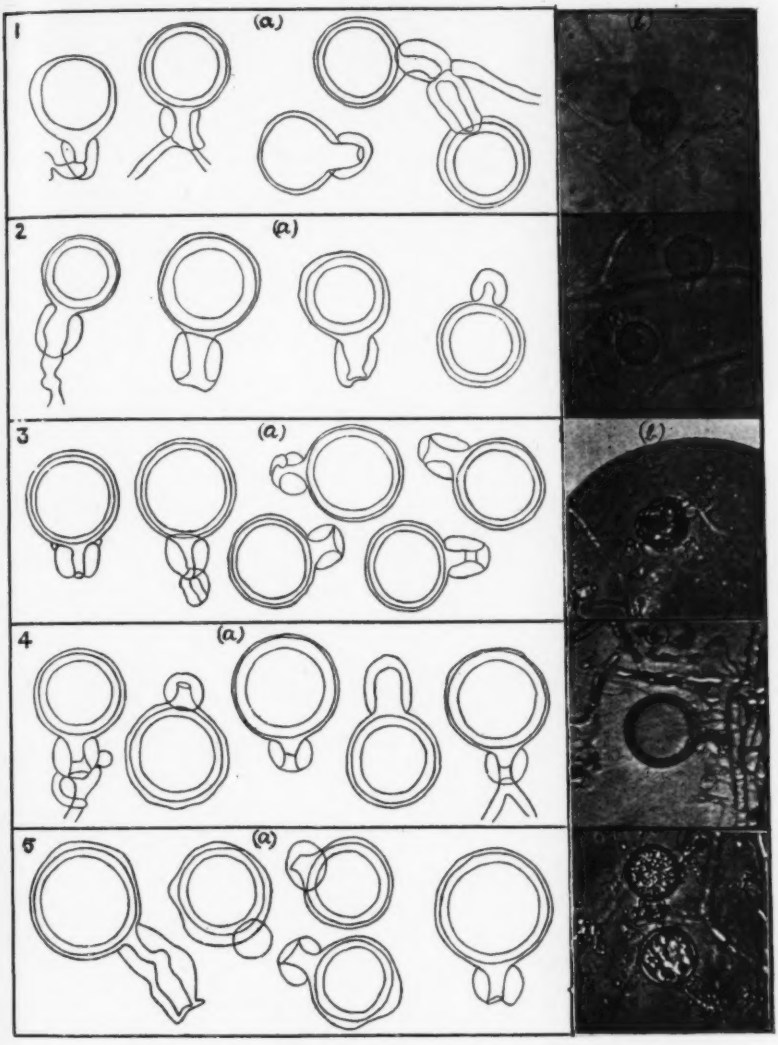
Three of these isolates belong to *P. palmivora* and the three others to *P. faberi*, *P. meadii* and *P. arecae* respectively.

P. arecae was found to produce oospores with *P. meadii*, *P. palmivora* and *P. faberi*.

P. arecae therefore falls into Gadd's " rubber " group and *P. meadii* into the " cacao " group.

Ashby's second grouping to accommodate *P. arecae* and *P. meadii* is superfluous.

The formation of oospores in combinations of *P. arecae* with *P. meadii* or any one of the members of the " cacao " group lends additional support for merging all these isolates as strains of *Phytophthora palmivora*.



OOSPORES IN PAIRED CULTURES

FIG. 1. *P. arecae* + *P. palmivora* (palmyra).
FIG. 2. *P. arecae* + *P. meadii*
FIG. 3. *P. arecae* + *P. palmivora* (orange).
FIG. 4. *P. arecae* + *P. faberi*.
FIG. 5. *P. arecae* + *P. palmivora* (coconut).

N.B.—(*a*) Camera drawings. × 345.
(*b*) Photomicrograph.

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THE CAUDAL VERTEBRÆ OF *ERYX JOHNII* (RUSSELL)

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The caudal vertebræ of the sand-snake *Eryx johnii* (Russell) show many important peculiarities which are unique in the whole order *Serpentes* (Ophidia). As they have not been dealt with by any previous worker, I am describing them in detail. Specimens for this study were collected at Agra. The skeletons were prepared by two methods: firstly, by maceration with caustic potash solution after two or three days' fixation in 95 per cent. alcohol, and secondly, by staining with alizarin and clearing in glycerin. For the sake of comparison, caudal vertebræ of *Python molurus*, *Ptyas mucosus*, *Naia naia* and *Lycodon aulicus* were also studied.

In all the snakes studied by me, the caudal region of the vertebral column can be divided into three sub-regions, more or less distinct from each other:

(*a*) *The anterior caudal sub-region*, situated immediately behind the pre-caudal[1] vertebræ. The vertebræ belonging to this sub-region are provided with bifurcated ribs or *processi costo-transversii* (*lymphapophyses* Salle, 1881) to protect the lymph-hearts, and vary in number from species to species or even within the same species, sometimes being numerically different on the right and the left sides of the same individual.

(*b*) *The middle caudal sub-region*, differing from the anterior caudal sub-region in the unforked nature of the ribs, and from the posterior caudal sub-region in the absence of hæmapophyses.

(*c*) *The posterior caudal sub-region*, comprising by far the largest number of caudal vertebræ. These have a pair of flat plate-like hæmapophyses on the ventral aspect of their centra and show a gradual reduction in size and in the development of their processes until they are represented near the tip of the tail by extremely short pieces with almost wholly vestigial processes.

In a specimen of *Python molurus* examined by me, there were 63 caudal vertebræ, of which the first four belonged to the anterior caudal sub-region, the next three to the middle caudal and the remaining fifty-six to the posterior caudal. Compared to *Python*, *Eryx johnii*, which also belongs to the same family (*Boidæ*), although to a different subfamily (*Boinæ*), shows a great

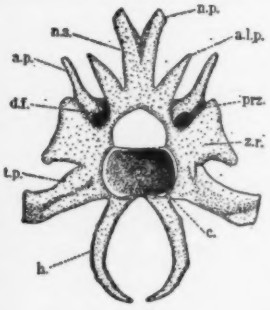
[1] The *pre-caudal* (*pre-sacral*) vertebræ can be readily distinguished from the *caudal* (*post-sacral*) by the form and direction of the ribs. While these are elongated rod-shaped structures sweeping distinctly backwards in the pre-caudal region, they are short, rather flattened and only slightly directed backwards in the caudal.

reduction in the number of the caudal vertebræ, the total number being only thirty-five. The anterior caudal sub-region comprises four vertebræ; the middle caudal, one; and the posterior caudal, thirty. Thus the reduction in the number of caudal vertebræ in *Eryx johnii* has affected only the middle caudal and the posterior caudal sub-regions, while the anterior caudal sub-region agrees with that of *Python molurus* in the number of vertebræ.

In addition to numerical reduction, the caudal vertebræ in *Eryx Johnii* show, as one proceeds backwards, a distinct diminution in their length as compared with those of other snakes. This feature as well as the reduction in the number of vertebræ is obviously due to a process of *shortening and compression of the caudal region* correlated with its transformation into a blunt and rounded, almost truncated, structure which has given rise to the popular misnomer "*Domuhi*" (two-headed) for the snake.

While the anterior caudal and the middle caudal vertebræ are similar in structure to those of other snakes, the posterior caudal vertebræ in *Eryx johnii* show many differences, not previously noted, from the typical structure of the vertebræ in snakes. The form of their neural spines, the absence of zygosphenes and zygantra, the development of a pair of accessory processes, the differentiation of the hæmapophyses into three types, and the presence of a few new foramina—all merit a detailed description. The hindmost two or three vertebræ are fused together to form a short, roughly rounded mass, covered with tubercle-like processes and supporting the caudal stump.

The neural spines of the first few posterior caudal vertebræ are single, but as we proceed backwards (Text-figs. 1, 2 and 4) they are bifurcated into



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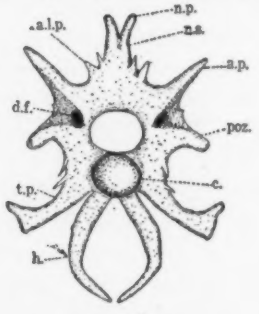

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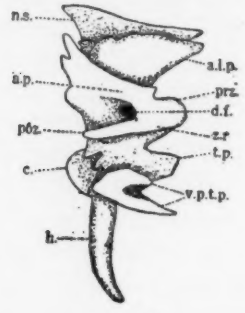
TEXT-FIG. 1. An anterior vertebra of the postero-caudal subregion: anterior view. × 4½.
a.l.p., accessory lateral process; *a.p.*, alar plate of the zygapophysial ridge; *c.*, centrum; *d.f.*, dorsal foramen; *h.*, hæmapophysis; *n.p.*, neural plate; *n.s.*, neural spine; *prz.*, prezygapophysis; *t.p.*, transverse process; *z.r.*, zygapophysial ridge.

TEXT-FIG. 2. An anterior vertebra of the postero-caudal sub-region: posterior view. × 4½.
poz., postzygapophysis (other abbreviations as in Text-Fig. 1).

two *neural-plates*, extending antero-posteriorly and diverging from each other more widely in the hinder vertebræ than in the anterior. Such a forking of the neural spines is a peculiarity not hitherto recorded in any snake. In a dorsal aspect the neural spines of the caudal vertebræ give an appearance of a single neural ridge dividing posteriorly into two lateral ridges. Each neural plate, viewed laterally (Text-fig. 3), slopes towards its anterior order,



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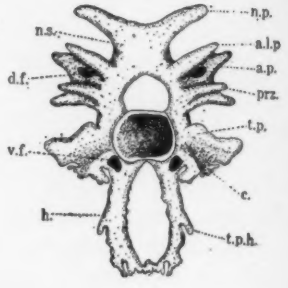

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TEXT-FIG. 3. An anterior vertebra of the postero-caudal subregion: lateral view. × 4½.
v.p.t.p., <-shaped plate of the transverse process (other abbreviations as in previous figures).

TEXT-FIG. 4. A posterior vertebra of the postero-caudal subregion: anterior view. × 4½.
t.p.h., tubercular prominences developed on the hæmapophysis; *v.f.*, ventral foramen (other abbreviations as in previous figures).

where it is produced into a small triangular process, fitting into a notch on the posterior border of the preceding neural plate.

The *zygosphenes* and *zygantra*, which occur in their normal relations in the caudal vertebræ of all other snakes, are present in *Eryx johnii* in only a few anterior vertebræ of the posterior caudal sub-region, but are absent in the posterior vertebræ. This is a unique condition, as no other ophidian vertebræ are so far known to lack these sructures.

Below the neural spine a pair of *accessory lateral processes* (Text-figs. 1-4), which are altogether new, occur in the form of obliquely directed plates at the sides of the vertebræ, each extending anteriorly into a projection which fits into a groove on the posterior face of the preceding vertebra between its accessory lateral plate and post-zygapophysis. In the anteriormost vertebræ of the posterior caudal sub-region, the accessory lateral processes co-exist, although in a less developed form, with the zygosphenes and zygantra —a fact showing that these plates are not modifications of zygosphenes but are new structures. In the posterior vertebræ the zygosphenes and zygantra are absent, while the accessory lateral processes are well-developed.

The *pre-zygapophyses* and *post-zygapophyses* (Text-fig. 3) are present, but are connected with each other at the side of the neural arch to form a lateral ridge inclined downwards posteriorly. A remarkable peculiarity is the presence of an accessory wing-like plate (*alar plate*), extending upwards and backwards from the middle of the *zygapophysial ridge*.

The *transverse processes* (Text-fig. 3) are well developed at the sides of the centra and are directed outwards and slightly downwards, but their appearance is very peculiar. Each transverse process projects at its outer aspect into the form of a <-shaped plate fitting into similar plates of the adjacent vertebræ. Such an articulation of the vertebræ by modifications of the transverse processes (*parapophysial articulation*) is hitherto unknown.

The *hæmapophyses* (Text-figs. 1, 2 and 4), unlike those found in other snake vertebræ, are of three kinds: firstly, in the anterior part of the posterior caudal sub-region they are in the form of short, smooth plates, projecting straight downwards; secondly, in the part where the neural ridge is bifurcated and also a little behind they present the appearance of rather smooth, curved pieces whose distal ends approximate each other, but remain separate; and thirdly, in the posteriormost part they fuse with each other to form a closed canal and have several tubercular prominences developed on them. Such a differentiation of the hæmapophyses is not known in any other snake.

Finally, two pairs of *foramina*, which are absent in the vertebræ of all other snakes as well as in the anterior caudal and middle caudal sub-regions of *Eryx johnii*, occur in its posterior caudal sub-region : firstly, a pair of *dorsal foramina*, enclosed by the alar plate and the bases of the accessory lateral processes and of the zygapophysial ridges (Text-figs. 1–3); and secondly, a pair of *ventral foramina* enclosed by the bases of the hæmapophyses and of the transverse processes (Text-fig. 4). While both these pairs of foramina are present in the posterior vertebræ, only the first pair is present in the anterior.

Summary

(1) The caudal region of the vertebral column in *Serpentes* can be divided into three sub-regions: *anterior caudal*, *middle caudal* and *posterior caudal*.

(2) The caudal part of the vertebral column of *Eryx johnii*, as compared to that of other snakes, shows a numerical reduction of the vertebræ, as well as a diminution in the length of their antero-posterior axes. This is due to a shortening and compression of the caudal region, mainly affecting the middle caudal and posterior caudal sub-regions.

(3) The posterior caudal vertebræ in *Eryx johnii* are unique in the whole order. They have bifurcated *neural spines*, are devoid of *zygosphenes* and

zygantra, possess *accessory lateral plates*, found nowhere else, have peculiar *alar plates* developed on the zygapophysial ridges, show parapophysial *articulation*, and are furnished with three types of *hæmapophyses*.

(4) Two pairs of foramina not recorded hitherto are found to occur in the posterior caudal sub-region of the vertebral column in *Eryx johnii*.

Acknowledgments

My best thanks are due to Mr. Beni Charan Mahendra not only for suggesting me the problem and translating for me the relevant German papers, but also for his constant encouragement and painstaking guidance.

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STUDIES IN THE GENUS *COLLETOTRICHUM*

II. Physiological Studies on *Colletotrichum falcatum* Went.

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Colletotrichum falcatum which causes the red-rot of sugarcane has a world-wide distribution being present in all countries where this crop is cultivated. In this paper are recorded the observations made on some aspects of the behaviour of the fungus in culture.

Materials and Methods

The fungus was isolated from a thick cane variety called *Poovan* which is usually subjected to heavy infestations of ' red-rot ' in Madras. The fungus was grown on solid and liquid media. Liquid media were synthetic and were employed to determine the dry weight of the fungus growth. 50 c.c. of the medium were placed in 100 c.c. Erlenmeyer flasks. After a definite period the growths were removed from the flasks, filtered through gooch crucibles and washed in several changes of distilled water. The crucibles were dried to constant weight by keeping in steam ovens. They were then ignited for 2–3 hours and the weights determined after ignition. The difference between the two weighings gave the dry weight of the fungus. The weight of the fungus ash being negligible was not taken into consideration. The cultures were run in triplicates and the average measurements taken. The inoculum consisted of equal quantities of spore suspensions or similar pieces of culture.

The Richards' solutions used in these experiments did not contain ferric chloride. The media were sterilised at 10 lbs. pressure for half an hour. pH determinations were made by the colorimetric method. The cultures were incubated at 26° or 29° C. Except where otherwise stated 150–200 spores were measured to arrive at average dimensions.

Sugarcane setts were inoculated in the following manner. The setts had three nodes. The ends were dipped in molten paraffin as soon as they were cut. Just above the lowest node a bore was made to 2/3 the thickness of the sett with a sterilised cork-borer. The inoculum was introduced into the hole and the plug replaced in position after cutting off a portion to make room for the inoculum. Melted paraffin was smeared over the plug. The

setts were then placed in washed moist sand and kept at laboratory temperature for a definite period. Afterwards they were taken out, split into two and the length and breadth of the reddened area measured. The virulence index was calculated by multiplying the ratio of lesion width to sett diameter by the ratio of lesion length to length of internode (Abbott, 1938).

The enzymes produced by the fungus were determined in different ways. The capacity of the fungus to grow on certain media was taken as an index of the formation of particular enzymes in the mycelium of the fungus (Uppal and Kulkarni, 1937). A stock medium containing magnesium sulphate ·5 gm., K_2HPO_4 1 gm., KCl 1·5 gm., ferrous sulphate ·01 gm., agar 20 gm. and distilled water 1000 c.c. was prepared. To this were added different compounds for testing particular enzymes. Another method followed was to grow the fungus in Richards' solution for two weeks and remove the fungus mats. These mats were then washed several times in sterile water, dried between filter-papers and then kept inside a desiccator for 10 days. The dried mat was then mixed with a small quantity of sterile water and silver sand and ground well in an agate mortar. The ground material was placed in 20 times the quantity of sterile distilled water, shaken well and allowed to stand for 24 hours at 15° C. The extract was filtered and some drops of toluene added to the filtrate (Venkatarayan, 1936). This extract was used for testing enzyme reactions and is referred to as 'crude enzyme extract'.

Growth on Media

The fungus grows well on a number of media and the nature of the growth on some of them is given below:

TABLE I

Growth of C. falcatum *on Different Media*

Medium	Initial pH	Diameter on 6th day in cm.	Nature of growth
French bean agar ..	4·7	6·75	Good, grey mealy aerial growth, zonation visible, numerous pink powdery masses of spores, setæ rare.
Quaker oats agar ..	4·5	6·52	Good, grey fluffy aerial growth with plenty of pink spore masses; black stromatoid bodies with setæ formed.
Richards' agar	4·5	4·4	Grey aerial growth less than above, numerous pink spore masses, black stromatoid bodies formed, setæ rare or absent.
Brown's agar	5·5	5·0	Thin growth, greyish white, aerial, pink spore masses less than in the above media, no setæ.
Soil-extract agar	6·25	3·72	Poor sparse growth, no spore masses or setæ.
Leaf-mould-extract agar ..	6·25	3·9	Do.

Quaker oats and french bean agars form the best media for the cultivation of the fungus; where a transparent synthetic medium is necessary Richards' agar may be used with advantage. The mycelium is made up of hyaline and brown hyphæ with numerous chlamydospores on the sides of the growths; but in Brown's, soil-extract and leaf-mould-extract agars the hyphæ are mostly hyaline with few chlamydospores. Setæ are rarely formed.

H-ion concentration of medium and growth.—The effect of different H-ion concentrations on the growth of the fungus was determined by growing the fungus in Richards' solution of different pH values at laboratory temperatures. The final pH of the media and the dry weight of the growth were determined.

TABLE II

Growth at Different H-ion Concentrations

Initial pH	pH after 23 days	Weight of fungus in gm.	Nature of growth
3·6	5·3	·1551	Grey, floating growth.
4·6	6·4	·3531	Thick grey growth, floating and submerged.
5·0	6·4	·2993	Do.
5·9	6·1	·1512	Less than the above.
6·6	6·6	·0827	Do.
7·4	6·8	·0292	Thin growth, light grey floating and submerged.
7·9	7·3	·0257	Little growth, mostly submerged.

The best growth occurs between pH 4·5 and 5·0, but the fungus is capable of growing over a wider range. Abbott (1938) noticed that the optimum was at 5·5 but he came to this conclusion from measurements of the diameters of growths on solid media. The pH is shifted differently according to the initial value but in most cases the final reading comes to be between pH 6 and 7.

The progressive shift in the pH value of the medium as a result of the fungus growth was determined at intervals by growing the fungus in Richards' solution of normal strength. Alongside with this, the effect of half strength of the medium on the growth and shift of pH was also noted.

In the first week the growth in the two strengths appear to be equal but from the second week onwards the growth in the normal solution is much more, almost double that in the half strength medium. But the shift in the pH is almost similar without relation to the strength of the solution or the amount of growth. After the second week the shift in the pH is not marked. After the third week there is a slight decrease in the weight of the mycelial growth in both the series.

TABLE III

Progressive Shift of pH Value of the Medium

No. of days' growth	Normal strength			Half strength		
	Initial pH	Final pH	Weight of fungus in gm.	Initial pH	Final pH	Weight of fungus in gm.
7	4·3	5·5	·0338	4·7	5·7	·0370
14	4·3	6·4	·2611	4·7	6·3	·1713
22	4·3	6·4	·5388	4·7	6·8	·2624
30	4·3	6·7	·5141	4·7	7·0	·2151

The filtrates were tested with Nessler's reagent when a brown precipitate was formed indicating the presence of ammonia in the solution. Boyle (1924) has stated that NH_3 is produced by some fungi during growth. The controls did not show any precipitate.

Temperature and Growth.—The fungus was grown in peptone agar in Petri dishes kept at different temperatures. The diameters of the growths were measured and the results are given below:

TABLE IV

Growth Characters of C. falcatum *at Different Temperatures*

Temperature in °C.	Diameter on 7th day in cm.	Average spore length in μ	Range in μ	Nature of growth
10	No growth	..	..	..
15	2·1	19·9	12–32	Thin white powdery growth, spores abnormal.
20	5·2	23·5	12–40	Thin whitish aerial and greyish olive submerged growth.
23	7·2	24·1	12–44	Pearl grey aerial growth; vinaceous fawn spore masses.
26	7·3	..	..	Pearl grey aerial browth with plenty of pale vinaceous fawn spore masses over most of the surface.
30	7·6	27·6	16–44	Pearl grey aerial growth with numerous shell pink spore masses over the whole surface.

From the above table it is found that 30° C. is the optimum among the temperatures tried. At 10° C. there was no growth but when the plates were removed and placed in the laboratory (about 28° C.) growth commenced and spread over the plate. The size of the spore is affected by temperature. The spore length increases from 15° C. onwards and at about 30° C. the normal size is attained. The shape of the spore produced at 15° C. is abnormally

curved. The intensity of sporulation increases with the temperature in the range studied.

Spores were kept in hanging drops at different temperatures for 24 hours and the correlation between temperature and percentage of germination and germ tube length was observed. 50 Measurements of germ-tube length were made.

TABLE V

Temperature and Germination of Spores

Temperature in °C.	Percentage of germination	Average germ-tube length in μ	Range in μ
10	..	..	..
15	5	76·4	40–108
20	50	138·4	52–216
26	82	163·5	120–280
32	100	191·5	160–338
37	84	71·8	24–128

Among the several temperatures tried, the percentage of germination and the growth of the germ tube in 24 hours are best at 32° C. Abbott (1938) has recorded 30–32·5° C. as the optimum for *C. falcatum*. Though germination at 37° C. is as good as at 26° C. the growth of the germ tube at the former temperature is much less. There is progressive reduction in germination and germ-tube length as the temperature falls below 32° C.

Humidity and spore size.—Infected sugarcane setts were kept exposed in the laboratory (about 60%) and inside moist chambers (100% humidity) for a week. Spores from the two series were measured to note the influence of humidity on spore length. The average spore size from the two series is the same ; those from setts exposed to laboratory air being 28·3 × 4·9 μ and those from setts inside the moist chamber 28·4 × 5·0 μ.

Slides containing air-dried spores were placed inside desiccators containing saturated solutions of $NaHSO_4$, $NaNO_2$, $NaClO_3$, KBr and Na_2SO_4, $7H_2O$ and distilled water in another desiccator capable of maintaining 52%, 66%, 75%, 84%, and 95% and 100% relative humidity respectively (Riker and Riker, 1936). When examined after 24 hours, germination was visible only in spores kept at 100% relative humidity and not in others.

Utilization of carbohydrates.—A stock medium containing KNO_3 10 gm., KH_2PO_4 5 gm., $MgSO_4$ $7H_2O$ 2·5 gm., and water 1000 c.c. was prepared. Two per cent. by weight of different carbohydrates were added to this solution in order to test the capacity of the fungus to utilise different sources of carbon. For the preparation of solid media 2% agar was added.

TABLE VI

Carbohydrates in Relation to Linear Growth of Fungus Colony on Solid Media

Source of carbon	Diameter in 12 days in cm.	Spore length		Remarks
		Range in μ	Average in μ	
Sucrose	8·0	16–40	28·12	Thick growth, soft woolly, pale olive grey, central portion raised, numerous pink spore masses.
Glucose	5·6	16–40	28·2	Pearl grey aerial growth not so thick as above, pink spore masses in the centre.
Starch (soluble) ..	7·2	16–40	29·96	Pale gull grey, soft fluffy, centre raised, not so thick as in glucose, pink spore masses in the middle.
Lactose	8·4	16–40	27·8	Less than in starch, a mixture of white, grey and chamois, soft plushy, spore production medium without prominent spore masses.
Maltose	4·2	..	..	Thin aerial, woolly chamois growth, spores scanty.
Cellulose	Not measureable	..	..	Very thin growth with few spores.
Arabinose	2·5	..	..	Very thin growth, light olive grey no spore masses.
No carbohydrate ..	Not measureable	..	..	Few radiating hyphæ.

TABLE VII

Carbohydrates in Relation to Growth in Liquid Media

Source of carbon	pH of control	pH after 18 days' growth	Dry weight of growth in gm.
Sucrose	4·3	6·8	·3234
Glucose	4·2	6·1	·1435
Starch	4·6	5·0	·0174
Maltose	4·4	5·3	·0115
Lactose	4·4	4·8	·0088
Cellulose	4·4	4·4	..
No carbohydrate ..	4·6	4·6	..

In both the series sucrose gives the best growth. Incidentally it is also brought out that linear measurements do not always give a correct picture of the amount of growth in media differing in composition, since it is not possible to differentiate between densities of growth. Lactose gives the largest diameter but the mat being very thin the quantity of growth is very poor. Being a parasite on sugarcane, it is natural that the best source of

carbon for this fungus is sucrose. Spore measurements show that there is no significant difference in the length of spores produced by injesting carbon from different sources. But there is marked difference in the intensity of sporulation sucrose producing profuse sporulation.

To the stock medium 2½%, 5%, 10% and 15% glucose and 2% agar were added to find out the effect of different concentrations of carbohydrate on the growth of the fungus.

TABLE VIII

Concentration of Glucose and Growth

Concentration %	Diameter in 8 days in cm.	Intensity of sporulation	Nature of growth
2½	8·8	+++	Good aerial greyish white growth; numerous pinkish spore masses.
5	9·0	++++	Best growth; more spore masses.
10	4·8	++	Less growth and less of spore masses.
15	4·6	++	Less growth and less of spore masses.

Sporulation is best at 5% and diminishes as concentration increases. The amount of growth also decreases with increase in concentration above 5%.

Utilisation of nitrogen sources.—The influence of varying amounts of nitrate in the medium, on the growth of the fungus, was investigated. Richards' solutions with 0, 5, 10, 15 and 20 gm. of potassium nitrate per 1000 c.c. were prepared. To these 2% agar was added.

TABLE IX

Different Amounts of Nitrate and Growth

Amount of nitrogen	Diameter in 9 days in cm.	Remarks
No nitrogen ..	10·0	Very thin light grey, with minute pink spore masses near margin; least sporulation.
5 gm. KNO_3 ..	10·2	Thick growth, aerial, mealy, olive grey; spore masses in patches of pink; stromatoid bodies many.
10 gm. „ ..	9·1	Thick, mealy, grey and white, spore masses in plenty in the centre; stromatoid bodies many.
15 gm. „ ..	8·6	Thick, mealy, light grey with numerous pink spore masses; profuse sporulation.
20 gm. „ ..	8·3	Not so thick as above; whitish with islands of grey growth; spores less profuse than in 10 gm.

In the medium with no nitrogen, the growth is very thin and the diameter is no measure of the amount of growth. The highest amounts of growth and sporulation were in dishes containing media with 10 and 15 gm. nitrate. The spore masses formed large pink patches over the growth.

The capacity of the fungus to utilise nitrogen from different sources was studied. A basic medium containing glucose 10 gm., KH_2PO_4 1·75 gm., $MgSO_4$. H_2O 0·75 gm., agar 15 gm., and water 1000 c.c. was prepared to which were added KNO_3, KNO_2, $(NH_4)_2SO_4$, urea, asparagin and peptone respectively as the different nitrogen sources. 5 gm. of KNO_3 were added to 1000 c.c. of medium. The other substances were used in quantities calculated to contain an equivalent amount of nitrogen.

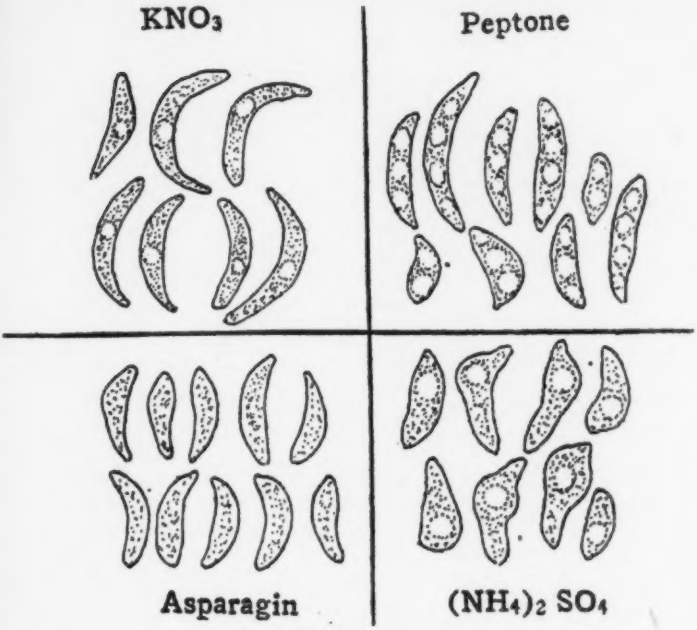
TABLE X

Different Sources of Nitrogen and Growth

Source of nitrogen	Diameter in 7 days in cm.	Mean length of spore in μ	REMARKS
KNO_3	2·2	31·4	Aerial growth pale gull grey, central portion thick; numerous shell pink spore masses forming patches.
Asparagin	4·6	24·4	Best growth, thick mineral grey to light mineral grey, irregular in outline; orange pink spore masses in plenty.
Peptone	7·8	27·6	Thinner growth, sparsely aerial; pearl grey with numerous shell pink spore masses.
$(NH_4)_2SO_4$	0·9	22·4	Extends little, pale olive grey and white; spores not many and abnormal.
Urea	0·9	..	Very thin growth, mostly submerged; no spore masses.
KNO_2	Nil	..	No growth.

Asparagin, peptone and KNO_3 are good sources of nitrogen. In ammonium sulphate there is an initial growth of the fungus which is quickly inhibited. Urea also forms a poor source. KNO_2 does not favour growth at all (Pl. XX, Fig. D). The nitrogen source influences the size of the spore and the intensity of sporulation. In $(NH_4)_2SO_4$ sporulation is limited and the spores are of peculiar shapes being bulged in the middle or otherwise malformed. In asparagin the spores are shorter than in potassium nitrate. Spores produced on peptone are more vacuolated than those of the same age formed on KNO_3 and asparagin (Fig. 1).

C/N ratio and growth.—Different proportions of carbon and nitrogen in Richards' solution and Richards' agar were used keeping the total weight of sugar and KNO_3 at 60 gm. per 1000 c.c. of medium. The different C/N ratios tried were 5/1, 2/1, 1/1, 1/2 and 1/5. On solid media, the best growth was on 5/1 closely followed by 2/1. The growth in the others were in the descending order with the least growth in 1/5. The medium in 1/2 and 1/5 was not completely solidified. In 5/1 and 2/1 a saltant developed. This was almost white, and was the predominant form in 5/1. While the olive



TEXT-FIG. 1

Spores from media with different nitrogen sources (× 500)

grey parental form was predominant in 2/1 (Pl. XX, Fig. E), in the other dishes the growth was olive grey without saltation. The growth was aerial and fluffy in all and spore formation was profuse. But the white saltant showed less of sporulation than the grey parent. At the junction of the two growths blackish stromatoid bodies were numerous. The two forms were isolated separately and in the following pages the white one is called light strain and the grey one, dark strain.

Liquid cultures of the different C/N ratios were also set up and the following table gives the dry weights of growths in these.

TABLE XI

C/N Ratio and Growth

C/N ratio	Initial pH	pH of control after 23 days	pH of inoculated after 23 days	Dry weight of fungus growth in 23 days in gm.
5/1	4·3	4·3	6·7	0·5276
2/1	4·4	4·3	6·3	0·4344
1/1	4·2	4·2	6·5	0·2644
1/2	2·9	2·8	6·5	0·1543
1/5	2·9	2·9	6·0	0·0946

The dry weight decreases as the C/N ratio decreases. The best growth is in 5/1.

Saltation.—Abbott (1938) mentions the occurrence of two distinct races of *C. falcatum*—light and dark—which may be distinguished by the colour and texture of the growth. He has also stated that *C. falcatum* is more or less stable and does not produce mutations. The Indian cultures of the fungus which he examined were morphologically similar to the dark race type of America. The present culture was started from a single spore. For over 10 months it maintained its original character and produced a saltant for the first time on Richards' medium as mentioned above. The production of the white strain as a saltant from the original dark stain throws light on how the light strains might have developed in nature as mutants from the dark one.

Comparative studies were made of the two forms. They were grown on Richards' and quaker oats agars and sterilised bits of cane. On both the agar media the distinction in colour is maintained. The dark form produces more spores than the light one. On sterilised cane bits the difference is more evident. The light strain develops a white growth at first which in course of time turns light grey. The other is dark grey from the start and maintains the colour.

The two forms were grown in liquid cultures kept at different temperatures to note whether there is any difference in their reaction to temperature.

TABLE XII

Temperature and Growth of the Two Strains

Temperature in °C.	Light strain Weight of 17 days' growth in gm.	Dark strain Weight of 17 days' growth in gm.
15	0·0802	0·0529
20	0·1292	0·1380
26·5	0·1965	0·4106
32·5	0·2146	0·4235
37	0·0055	0·0020

Both the forms thrive best at 32°·5 C. The dark form produces more growth at the optimum temperature.

The thermal death point of the spores of the two strains was determined. Fine capillary tubes 2″ in length and closed at one end were placed in spore suspensions and kept under an air pump. On removal they were filled with the liquid containing spores. Six tubes were fastened to the stem of a thermometer with rubber rings and then immersed in sterile water, kept at a constant

temperature for 5 minutes or ten minutes (Thomas, 1934). After removal from the water, the tubes were placed in Petri dishes containing media and crushed by means of sterilised forceps to liberate the spores. Observations were made on the germination of spores after 24 hours.

TABLE XIII

Thermal Death Point

Temperature in °C.	Time in minutes	Light strain	Dark strain
52	5	No germination	No germination
51	10	do.	do.
51	5	do.	do.
50	10	do.	do.
50	5	Germination	Germination
49	10	do.	do.
49	5	do.	do.

The spores are killed in both the strains by 5 minutes exposure at about 51° C. But chlamydospores are able to germinate after 5 minutes exposure at this temperature.

The relation between temperature and the rate of spread of infection as shown by the reddening of the internal tissue was studied with setts of Co. 419. After inoculation the setts were placed in basins along with moist cotton wool and kept inside incubators at different temperatures. Top setts and bottom setts were inoculated. The following table gives the virulence index (Abbott, 1938) in 14 days.

TABLE XIV

Virulence Index at Different Temperatures

Temperature in °C.	Light strain		Dark strain	
	Bottom setts	Top setts	Bottom setts	Top setts
15	0·19	0·86	0·03	0·11
20	0·29	1·00	0·18	0·34
26	0·39	1·00	0·30	1·00
30	0·46	1·00	0·39	1·00
34	0·80	1·00	0·23	0·56

In the controls the red colour was confined to the immediate neighbourhood of the bores and there was no spread. Infection spreads quicker in the top setts. The maximum infection by the dark strain is at about 30° C. and by the light strain at about 34° C.

Three varieties of canes—Co. 213, Co. 413 and Co. 421—were inoculated with the two strains for comparing their pathogenic capacities. Below are given the virulence indices at the end of 18 days.

TABLE XV

Virulence Index

Variety	Dark strain	Light strain
Co. 213 ..	0·67	0·54
Co. 421 ..	0·62	0·78
Co. 413 ..	0·61	0·62

The pathogenecity of the two strains varies in certain varieties.

Antagonism.—During the course of studies it was found that when the cultures were contaminated by a dark green *Aspergillus*, the growth of *C. falcatum* was arrested. This was common to both strains. It was presumed that this may be due to formation of some staling products by *Aspergillus* which inhibited the growth of *Colletotrichum*. To confirm this the dark strain was grown on media containing filtrates of *C. falcatum*—both strains, *C. indicum* and the *Aspergillus* sp. These fungi were first grown in Richards' solution for 16 days. The media were then filtered and the filtrates mixed with equal quantities of fresh Richards' solution. In the control equal quantities of water and Richards' solution were used. After sterilisation the media were inoculated with the dark strain. The dry weight of the fungus was determined after 17 days.

TABLE XVI

Growth on Filtrates of Other Fungi

Medium	Dry weight in gm.
Filtrate of *C. falcatum* (dark)	0·2615
Do. (white)	0·2768
Filtrate of *C. indicum*	0·2537
Do. *Aspergillus*	0·0203
Control	0·1147

The filtrates of *Colletotrichum* have no inhibitory effect on growth. But the filtrate of *Aspergillus* reduces growth to a large extent, the effect being persistent even after autoclaving.

Trichoderma lignorum was grown along with the two strains of *Colletotrichum falcatum*. *T. lignorum* and strains of *Colletotrichum* were inoculated

in two places in the same dish. In five days *T. lignorum* had grown over the growth of the dark strain. The light strain was surrounded and was just beginning to be overgrown (Pl. XX, Fig. A). In 10 days the light strain also was overgrown. In both cases the *Colletotrichum* hyphæ had disintegrated in the portion overgrown by *T. lignorum* though there was no evidence of penetration of the hyphal cells by *Trichoderma* (Pl. XX, Figs. F & G). The dark strain is more easily affected than the light strain.

The growth of *Colletotrichum* in the filtrate of *Trichoderma* was tested. *T. lignorum* was grown in Richards' solution for 15 days. The solution was then filtered. Three series of media were prepared, on containing equal quantities of the filtrate and Richards' solution, another Richards' solution alone and a third with equal quantities of distilled water and Richards' solution. The dry weights of the fungi were determined after 17 days' growth.

TABLE XVII

Trichoderma Filtrate and Growth

Medium	Light strain in gm.	Dark strain in gm.
Trichoderma filtrate *plus* Richards' solution	0·2049	0·2669
Richards' solution	0·2780	0·3843
Richards' solution *plus* water ..	0·1720	0·2216

The autoclaved filtrate does not appreciably inhibit the growth of the two strains of *C. falcatum*.

Enzymes.—Some work on enzyme production by *C. falcatum* has been carried out by Lewton Brain (1908) in Hawaii. The production of invertase and very restricted quantities of cytase have been noted. Experiments were conducted with the Madras strain of *C. falcatum* and its saltant to observe the different kinds of enzymes produced.

Invertase.—This was investigated by growing the fungus in autoclaved sugarcane juice for one week and estimating the relative amounts of sucrose and glucose in the filtrate.

TABLE XVIII

Inversion of Sucrose

Medium	pH	Glucose %	Sucrose %
Control	4·7	1·64	16·27
Inoculated and one week old	6·1	6·52	11·21

The increase in the percentage of glucose with a decrease in sucrose contents in the inoculated juice shows the activity of the invertase produced by the fungus. One c.c. of the crude enzyme extract was mixed with 9 c.c. of 2% sucrose solution. Toluene was added. The solution was tested after 6 hours with Fehling's solution. There was no precipitate in the control while a deep red precipitate was formed in the tubes to which the extracts had been added. Both the strains produce invertase.

Diastase.—Two per cent. soluble starch was added to the stock medium. After 8 days of fungus growth the dish was flooded with 1% iodine solution. A small area one inch in diameter in the centre of the growth remained unaffected while the rest of the medium turned dark blue. This indicates that small quantities of diastate are formed.

One c.c. of the crude enzyme extract was added to 9 c.c. of 0·5% solution of soluble starch and tested with Fehling's solution after 24 hours. Both the strains showed the formation of precipitate, but the dark strain produced a deeper coloured precipitate showing greater enzyme activity. There was no change in the control.

Pectinase.—Potato discs 1 mm. in thickness were kept in the enzyme extracts of both the strains, boiled extracts and in sterilised water. In three hours, the discs kept in the unboiled extract of the dark strain became limp and could be easily torn while it took 4 hours to reach this condition in the extract of the light strain. In water and the boiled extracts the discs remained turgid. The limpness and the quick tearing of the potato discs show the presence of a comparatively higher pectinase content in the extract of the dark strain (Brown, 1915).

Oxidase.—Bavendam's method (Venkatarayan, 1936) was used for the determination of oxidase by growing the fungus in potato dextrose agar with 0·25% and 0·5% tannic acid. In the tannin agars, a dark brown halo developed round the growths. This was absent in the controls (Pl. **XX**, Fig. C). The halo increased with the time and was in advance of the mycelial growths. The growth of the fungus was much less in tannin agars compared to the control. The growth decreased with the increase in tannin contents. The tannin is utilised by the fungus by the production of oxidase.

Trypsin.—Egg albumen agar (Uppal and Kulkarni, 1937) was used. The light strain produced a thin growth and the medium cleared to some extent under the growth showing the utilisation of the coagulated albumen. The dark strain produced a poorer growth and the clearance of the medium was much less. The presence of small amounts of trypsin in the mycelium is indicated by the partial clearance of the medium.

Amidase.—This was determined by growing the fungus on asparagin-rosolic-acid agar. The white strain produced poor aerial growth but the medium was reddened on the 4th day. The dark strain produced less growth and changed the colour of the medium to red only on the 8th day. The white strain produces more amidase than the dark one.

Lipase.—Litmus cream agar was used to test the presence of this enzyme. Both the strains produced medium aerial growth and the colour of the agar was changed into red. This reddening must be due to the formation of fatty acids produced by the action of lipase on fats.

Erepsin.—The two strains were grown on casein agar. It was found that in the dishes containing the light strain the medium became clear under the growth due to the digestion of casein, by the production of erepsin. The medium in which the dark strain was growing was cleared to a slight extent.

Emulsin.—Salicin agar was inoculated with both the strains. The light strain produced a thin growth 2 cm. in diameter in 12 days, while the dark strain did not grow at all. Hence it is concluded that the light strain produces very little emulsin and the dark one none at all.

Inulase.—Inulin agar was used to test the presence of inulase in the mycelium. Both the strains produced thin aerial growth showing that inulase is produced by them and hence they are able to digest inulin.

The enzyme studies show that the light and the dark strains produce plenty of invertase. Inulase, lipase and oxidase are formed in equal amounts, in both. The dark strain produces more of diastase and pectinase and the light strain more of trypsin, amidase and erepsin. Emulsin is not produced by the dark strain while the light strain forms small quantities of this.

General.—Abbott's (1938) observations from a study of numerous isolates showed that *C. falcatum* remained stable morphologically and that 'sectoring' did not appear in the cultures. In the course of the experiments described in this paper the cultures were true to the original isolate except on one occasion when sectoring developed in Richards' agar giving rise to a dark and a light form. In the beginning there was difference in spore production between the two strains, the dark one producing more spores than the light one. But spore formation after a number of sub-cultures is not of the same original intensity. The pink masses are very rare after 26 months in culture. Atkinson (1938) has observed the cessation of sporulation in culture after some time. The spores are in the earlier cultures of the isolate in pink masses produced from hyphal branches. Some times stromata are formed and these bear one or two setæ. These may rarely bear spores at

their apices (Fig. 2). Appressoria develop in large numbers in both the strains being more profuse in the dark strain. Sporulation in the old cultures of the dark strain can be improved by growing the fungus on sterilised sugarcane bits.

TEXT-FIG. 2
Seta producing spore

Acknowledgments

The writer's thanks are due to Mr. P. V. Ramiah, Government Agricultural Chemist, for kindly arranging the analysis of sugarcane juice and for affording facilities for work in his laboratory and to Mr. K. M. Thomas, Government Mycologist, for reading through the manuscript and for helpful criticism during the course of the work.

Summary

C. falcatum grows well on a number of media. The best growth is formed between pH 4·5 and 5. About 32° C. is the optimum temperature for growth. The pores are killed by an exposure of 5 minutes at about 51° C. The spore length is affected by the temperature at which the fungus grows.

Sucrose is the best source of carbon. The source of carbon has no influence on spore length. But the intensity of sporulation is affected.

Asparagin, KNO_3 and peptone are readily utilised by the fungus. The growth is poor on $(NH_4)_2SO_4$ and urea. There is no growth in KNO_2. The spore length and intensity of sporulation are influenced by the particular nitrogen compounds used. The best growth occurs when C/N ratio is 5/1.

A light coloured saltant was formed. The two strains have similar temperature ranges and thermal death points. But they exhibit differences in their sporulation, pathogenicity, amount of growth and colour.

Growth is inhibited by *Trichoderma lignorum* in paired cultures, but the autoclaved extract has no inhibitory influence. An *Aspergillus* sp. inhibits the fungus in culture and its autoclaved filtrate has also a similar effect.

A number of enzymes are produced by the two strains.

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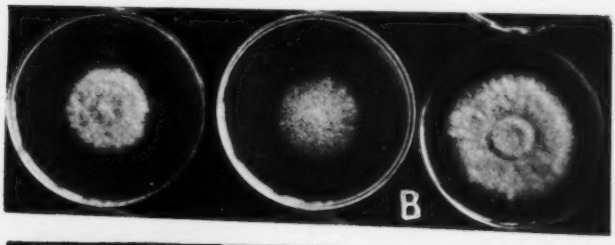
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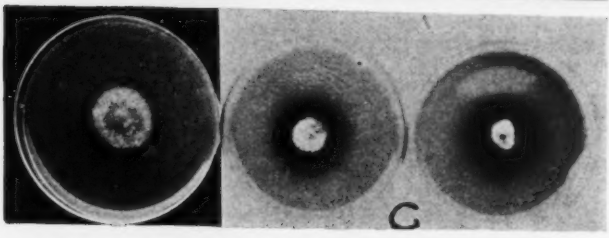
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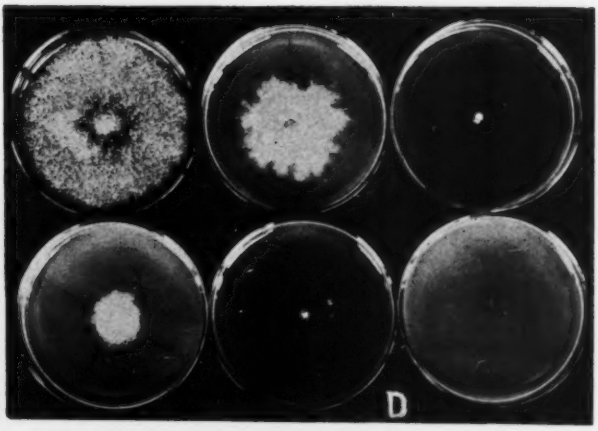
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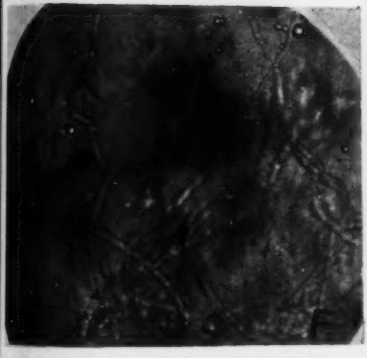

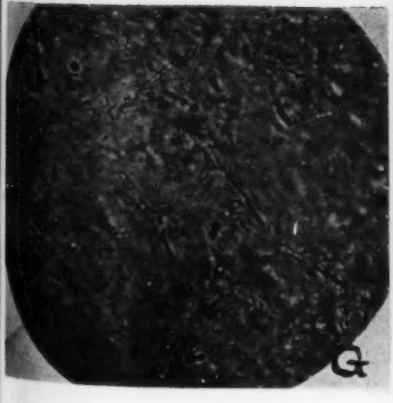
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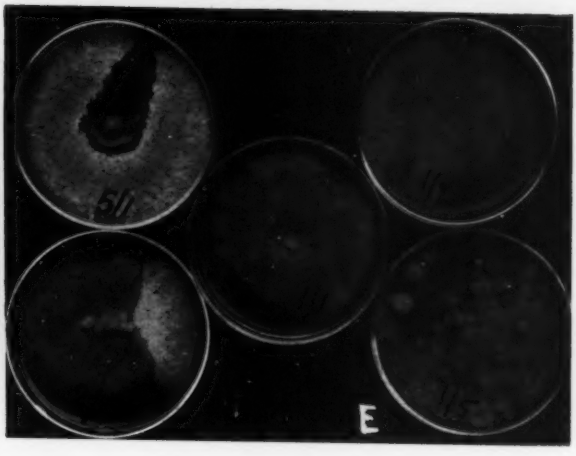
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EXPLANATION OF PLATE XX

A. Paired cultures of *Trichoderma lignorum* and light strain (1) and dark strain (2) of *C. falcatum*.

B. Growth of *C. falcatum* on Richards', Brown and French bean agars (left to right).

C. Growth on potato dextrose agar (extreme left). ·25% Tannin agar (middle) and ·5% tannin agar (right). The dark halo is visible in the tannin agars.

D. Growth on different nitrogen sources (top row, left to right). Peptone, Asparagin and urea (bottom row, left to right). KNO_3 $(NH_4)_2SO_4$ and KNO_2.

E. Growth on different C/N ratios.

F. Hyphæ of *C. falcatum*.

G. Hyphæ of *C. falcatum* disintegrated by *T. lignorum*.

STUDIES IN RUBIACEÆ

II. *Spermacoce hispida* Linn., *Guettarda speciosa* Linn. and Some Cytomorphological Considerations

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I. Introduction

Previous work in the family has been summarised by Raghavan and Rangaswamy (1941). The genus, *Spermacoce*, has not been worked out in detail by the previous workers, though the related genus *Diodia* has been studied to some extent.

Lloyd (1902) mentions that in *Spermacoce* the embryo-sac is slender and cylindrical. Fagerlind (1937) reports the presence of strophiole and a reduced type of nucellus. He classes the embryo-sac of *Spermacoce* under the *Richardsonia* type. None of the workers has investigated the genus *Guettarda*.

The present communication deals with the development of microsporangium, embryo-sac and embryo in *Spermacoce*. Some details of meiosis

in *Spermacoce* and *Guettarda* are described. The embryo-sac and the embryo development in *Guettarda* have also been described.

II. Materials and Methods

Materials of *Spermacoce* and *Guettarda* were available in plenty locally. For meiotic stages, the buds in the case of *Spermacoce*, and the anthers in the case of *Guettarda*, were fixed in Navaschin's fluid after prefixation in Carnoy's fluid. The required stages for fixation were determined through aceto-carmine examination.

Stages of microsporangium development and early stages of megasporogenesis for *Spermacoce*, were obtained from small buds fixed in formalin-acetic-alcohol. For the former, transverse sections were taken and for the latter, longitudinal sections were cut. For stages upto eight-nucleate embryo-sac, whole ovaries were fixed. For post-fertilization stages, ovules were taken out and fixed.

In the case of *Guettarda*, ovaries were fixed after removing the pubescent coating by means of a sharp blade. Because of the irregular orientation of ovules, many sections had to be cut before the required stages were obtained. Ovules were dissected out and fixed separately for post-fertilization stages.

The materials were dehydrated in alcohol and imbedded in paraffin wax using chloroform as the paraffin solvent. Sections were cut at thicknesses varying from 6 to 8 microns and stained in iron-alum hæmatoxylin and iodine gentian-violet.

III. Spermacoce hispida Linn.

(*a*) *Microsporangium development and Microsporogenesis.*—A transverse section of a very young anther shows at each of the four corners a hypodermal band of two primary archesporial initials (Figs. 1 and 2). Through the periclinal division of these cells, two layers of cells are formed, the outer being the primary wall layer and the inner, the primary sporogenous layer. The former divides and gives rise to more wall layers, while the latter gives rise to the sporogenous tissue (Fig. 3). In the present case, the sporogenous tissue is formed only to a limited extent. At the mature stage the wall of the anther sac consists of only three layers of cells (Fig. 4).

In addition to the three wall layers, a tapetal layer is differentiated from the innermost layer of the wall cells (Fig. 4). This consists of cells which during early stages are full of cytoplasm and are uninucleate (Fig. 5). Later they come to possess two nuclei (Fig. 6). This takes place by ordinary mitotic

division. Another interesting phenomenon is the fusion of two or more nuclei, resulting in a single nucleus with more than one nucleolus. Fig. 7 shows a binucleolated nucleus which is the result of fusion of two ordinary nuclei. Fig. 8 shows a tapetal cell, whose nucleus has three nucleoli, possibly from the fusion of a two nucleolated nucleus with an ordinary nucleus. Nuclei having more than three nucleoli were not found. Such a fusion of tapetal nuclei has been recorded in *Gynandropsis* (Raghavan, 1938) and in *Nicotiana glutinosa* (Raghavan and Srinivasan, A. R., 1941 *b*).

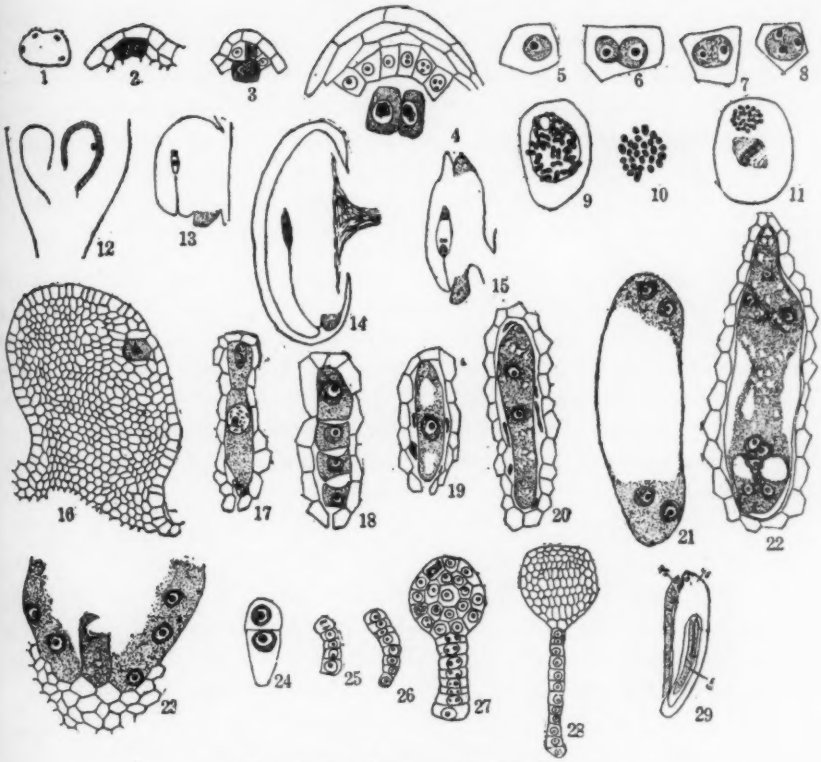
During prophase the pollen mother cells round themselves off and are very big in size. Owing to the slender nature of the chromatic threads and the comparatively large number, the details of synapsis before diakinesis could not be studied. At diakinesis 28 bivalents were counted, most of which were rod-shaped (Fig. 9). According to Nandi (1936) rod-shaped bivalents result from the small chromosomes, with one chiasma in one arm. Lawrence (1931) observes that the rod-shaped type is characteristic of small-chromosomed plants. The other bivalents are probably of the ring type.

Prometaphase follows diakinesis and the nucleus enters upon the first metaphase stage (Fig. 10). 28 Bivalents were counted of which three are somewhat bigger than the rest. Probably these three are the three ring bivalents observed during diakinesis. All the bivalents lie closely packed in the equatorial plate. This confirms the previously reported haploid chromosome number of the plant by Raghavan and Rangaswamy (1941).

The disjunction of bivalents is normal and at M II (Fig. 11). 28 Chromosomes could be counted at each pole of the P.M.C. The pollen grains are formed usually tetrahedrally. The pollen grains are comparatively large.

(*b*) *Development of embryo-sac and embryo.*—The ovary is two-carpelled and bilocular containing in each locule a solitary amphitropous ovule. The ovule at first arises as a straight protuberance from the base of the ovary (Fig. 12). The archesporium is differentiated laterally in a hypodermal position in the ovule. As the ovule grows and bends to assume the amphitropous configuration, the megaspore mother cell comes to point towards the base of the ovary. The ovule has a single massive integument (Fig. 13).

As the ovule bends to form the amphitropous structure, an outgrowth of the funicle is seen to develop on the side of the ovule towards the funicle (Figs. 13–15). This was termed the "strophiole" by Lloyd (1902) who found it in *Diodia*. It occurs in a number of other genera belonging to Coffeiodeæ such as *Richardsonia*, *Leptodermis*, *Pavetta*, *Ixora*, etc., and also in *Cephalanthes*, a genus belonging to Cinchonoideæ. A similar structure in



FIGS. 1–29. *Spermacoce hispida* Linn.

FIG. 1. Transverse section of a young anther. × 150. FIG. 2. Hypodermal band of two archesporial cells. × 750. FIG. 3. Formation of primary wall layer and primary sporogenous layer. × 750. FIG. 4. Mature anther with three wall layers, the tapetum and the pollen mother cells. The nuclei of the tapetum are multicleolated. × 750. FIG. 5. Uninucleate tapetal cell. × 1500. FIG. 6. A binucleate tapetal cell. Nuclei are in the process of fusion. × 1500. FIG. 7. A tapetal cell with a nucleus which has two nucleoli. × 1200. FIG. 8. Tapetal cell whose nucleus has three nucleoli. × 1200. FIG. 9. Diakinesis in P.M.C. × 2200. FIG. 10. First metaphase. × 2200. FIG. 11. Second metaphase. × 2200. FIG. 12. Longitudinal section of an young ovary showing the origin of the ovules. × 500. FIG. 13. Ovule showing the megaspore mother cell, integument, micropylar canal and the strophiole. Strophiole is represented by the dotted portion. × 150. FIG. 14. Ovule at the time of binucleate embryo-sac stage. × 75. FIG. 16. Archesporium of the megaspore. × 500. FIG. 17. Megaspore mother cell. × 750. FIG. 18. Linear tetrad. × 750. FIGS. 19–21. Uni-, bi-, four-nucleate embryo-sacs. × 750. FIG. 22. Mature embryo-sac. × 500. FIG. 23. Zygote and nuclear endosperm. × 500. FIG. 24. Two-celled proembryo. × 500. FIGS. 25 & 26. Linear proembryos of 5 and 8 cells. × 150. FIGS. 27 & 28. Mature embryos. × 350; FIG. 29. Section of a mature seed. × 10. *In.*, Integument; *Em.*, Embryo; *St.*, Strophiole.

Coffea has been termed as *obturator* by Howk (1938). Whatever its name may be, its function is not definitely known. Lloyd (1902) attributes to it a nutritive function and thinks that "it may be the seat of some metabolic activity during the growth of the embryo and the endosperm".

From the position it occupies (Fig. 15) there is much room to think that it controls in some way the ingress of the pollen-tube into the micropylar canal. But this can be substantiated only after critical pollination and fertilization studies.

The archesporium is enveloped by two or three cells which form the nucellar epidermis. As the megaspore mother cell enlarges, the two nucellar cells are crushed, caught as they are between the massive integument and the enlarged megaspore mother cell (Fig. 17). The exact type of nucellus in the present case could not be made out. But the figures of Fagerlind (1937) show very well that a straight row of cells cover the archesporium upto the linear tetrad stages in *Spermacoce tenuior*, and he regards it as belonging to the *Vaillantia*-type.

The archesporium is differentiated from the hypodermal layer. It consists of a single cell. The cells near the archesporium are somewhat elongated (Fig. 16). Fig. 17 represents a megaspore mother cell and another cell towards the chalazal end. This is also shown in Fig. 13, as occurring just below the megaspore mother cell. Presumably this is an additional megaspore mother cell situated below the normal one which, after some time, is arrested in its development and functions as any other ordinary cell of the surrounding integument. Multicellular archesporium is not rare in this family. Though not common in this genus, other genera like *Galium*, *Callipeltis*, etc., have been known to exhibit two or more megaspore mother cells, each of which was found to develop into the linear tetrad in the normal manner (Lloyd, 1902).

The megaspore mother cell gives rise to the linear tetrad of megaspores (Fig. 18). The chalazal megaspore grows into the uninucleate embryo-sac (Fig. 19). The uninucleate embryo-sac develops into the mature embryo-sac according to the monosporic type of embryo-sac development (Maheshwari, 1937) (Figs. 20–22).

The growth of the embryo-sac is very rapid and the size of the mature embryo-sac is very large (Fig. 22). The latter is cylindrical, tapering at the chalazal end. The egg apparatus consists of well-defined synergids and egg cell. The polar nuclei which are about to fuse lie closely adpressed to the egg cell. The most remarkable feature is the nature of the antipodals. The antipodals are very large with prominent nuclei. The lowermost of the three

antipodal cells has a tapering end and is the biggest. The antipodal apparatus bears a great resemblance to the early stages in the development of antipodal haustonia which occur in many genera of this family (*e.g.*, *Callipeltis*, *Asperula*, *Diodia*, etc.) (Lloyd, 1902). In this case however, they degenerate after fertilization.

The fertilized egg divides transversely and develops into the proembryo (Fig. 24). The endosperm nucleus divides by free nuclear division and gives rise to nuclear endosperm (Fig. 23). Cell wall formation in endosperm tissue takes place later.

Through repeated divisions, an eight-celled linear proembryo is formed (Figs. 25 & 26). Further development appears to be normal. One important feature of this species is the unusual length of the suspensor which is made up of 12 cells at the mature stage. The suspensor is uniseriate (Figs. 27 & 28). Such a long uniseriate suspensor is met with in *Leptodermis* (Fagerlind, 1937) and in *Diodia* (Lloyd, 1902).

The mature seed (Fig. 29) consists of the remains of the strophiole (*St*) and the thick integument (*In*), the latter of which does not perish as in *Dentelal* and *Oldenlandia*. The cells of the integument become thick-walled and the seed thus is very hard ultimately. There is no endosperm surrounding the embryo (*Em*).

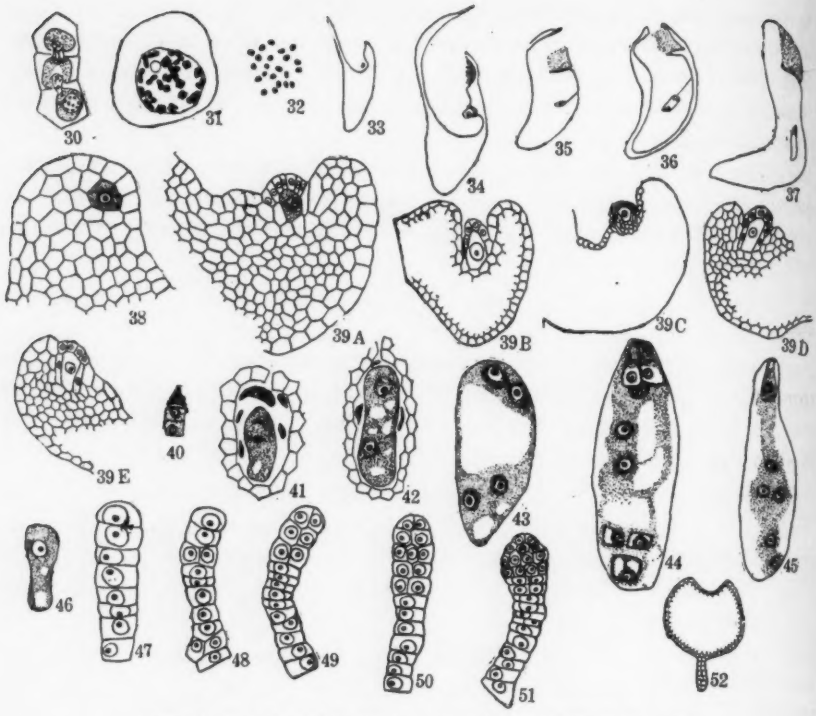
IV. Guettarda speciosa Linn.

(*a*) *Microsporogenesis.*—The prophase studies in the P.M.C. of *Guettarda* occasionally show the interesting phenomenon of cytomyxis (Fig. 30). Cells exude their nuclear contents into adjacent cells. Such a feature has been found characteristic of interspecific hybrids and has been observed in the case of the hybrid between *Nicotiana tabacum* × *N. glutinosa* (Raghavan and Srinivasan, A. R., 1941*a*.) It has also been found in *Tridax procumbens* (Raghavan and Venkatasubban, 1941). Among the three cells, in the first cell, extrusion takes place through three passages and in the other cell through a single passage. The phenomenon occurs only rarely and does not affect the meiotic regularity of the plant.

During diakinesis, 22 bivalents are observed. The rod and the ring types of bivalents are found (Fig. 31).

During metaphase, the 22 bivalents arrange themselves in the equatorial plate. They do not lie compactly as in the case of *Spermacoce*, but lie dispersed in the equatorial plate.

The rest of the meiosis takes place normally with the production of good viable pollen.



FIGS. 30–52. *Guettarda speciosa* (except Figs. 39 B, C, D and E)

FIG. 30. Cytomyxis in pollen mother cells. × 700. FIG. 31. Diakinesis. × 2200. FIG. 32. First metaphase. × 2200. FIG. 33. Ovule and ovarian chamber, when archesporium is just differentiated. × 150. FIG. 34. Ovule and ovarian chamber when integument is differentiated. × 150. FIG. 35. Ovule at the time of uni-nucleate embryo-sac. × 75. FIG. 36. Ovule during four-nucleate embryo-sac. × 75. FIG. 37. Mature ovule, in which a mature embryo is formed. × 10. Strophiole shown stippled in Figs. 34–37. FIG. 38. Archesporium. × 750. FIG. 39 A. Showing nucellus, archesporium and integument. × 750. FIG. 39 B. Ovule of *Ophiorrhiza* showing a convex layer of nucellus consisting of five cells. × 750. FIG. 39 C. Nucellus of *Canthium*. × 355. FIG. 39 D. Nucellus occurring in *Mussaenda*. × 750. FIG. 39 E. The nucellar epidermis of *Pentas*. × 750. FIG. 40. Linear tetrad with the two micropylar cells degenerated. × 750. FIGS. 41–43. One-, two-, and four-nucleate embryo-sacs. × 750. FIG. 44. Mature embryo-sac. × 750. FIG. 45. Oospore and endosperm nuclei. × 500. FIG. 46. Oospore. × 750. FIGS. 47–52. Embryo stages. × 750. FIG. 52. Lobing of cotyledons in the embryo. × 150.

(*b*) *Embryo-sac and embryo development*.—The ovary is syncarpous consisting of 7 to 9 carpels and as many locules. The ovules are solitary and pendulous. They first arise as pendulous protuberances in the carpel chamber (Fig. 33). The archesporium is differentiated on the free end of the

ovule and points downwards. As the ovule grows, it curves along its long axis and the archesporium comes to assume a horizontal configuration (Fig. 34). The single integument is now differentiated and it grows into a massive structure, so that the archesporium comes to lie deep in the ovule. The micropylar canal is formed. Still later the ovule curves more, causing the embryo-sac and the micropylar canal to form an acute angle with the long axis of the ovule (Figs. 35 & 36). After the four-nucleate embryo-sac stage, the part of the ovule at the antipodal end elongates and curves away from the side in which the embryo-sac is situated. The result is that the mature ovule is not straight, but is bent. The embryo-sac comes to be situated during this stage, just at the portion of the bend (Fig. 37).

The funicle is thick. The growth of the integument results in the formation of the micropylar canal. When the integument is differentiated, the strophiole develops as an outgrowth from the funicle (Fig. 34). Its growth is soon arrested and it is seen in later stages as an outgrowth of the funicle capping the ovule at its basal portion. The strophiole in this case is not so very prominent as in *Spermacoce* (Figs. 35, 36 & 37). It could be referred to as merely the funcile but for the fact that it is not connected to the rest of the ovular body as the funicle should be.

The archesporium differentiated from the hypodermal layer, consists of a large single cell with a prominent nucleus (Fig. 38). At later stages the integument is differentiated (Fig. 39 A). With the growth of the integument, the archesporium comes to occupy a deep position in the ovule. There it elongates into the megaspore mother cell, which undergoes meiosis and results in the linear tetrad. Of the tetrads, the two micropylar cells degenerate soon, and the third degenerates only slowly. The chalazal megaspore develops (Fig. 40).

When the archesporium is differentiated and the integument arises, the former is covered by seven cells which form the somewhat convex nucellar epiderms (Fig. 39 A). This bears a great resemblance to the nucellus of *Phyllis*. So the nucellus in the present case belongs to the primitive *Phyllis*-type. With the development of the integument, the nucellar layer is crushed.

Due to the growth of the megaspore into uninucleate embryo-sac, the integumental cells in the neighbourhood of the embryo-sac disintegrate (Fig. 41). The nucleus of the uninucleate embryo-sac divides and the daughter nuclei travel to opposite poles. Fig. 42 shows the two-nucleate embryo-sac. The rapid growth in size of the embryo-sac results in the further degeneration of the immediately surrounding integumental cells (Fig. 42). By further division of the nuclei the four- and eight-nucleate embryo-sacs are

formed (Figs. 43 & 44). The mature embryo-sac has the synergids and the egg cell at the micropylar end. At the chalazal end there are three antipodals. The antipodals are of normal size and all the three are of the same size unlike those of *Spermacoce*. The polar nuclei are found in the centre of the embryo-sac (Fig. 44) and later fuse to give the secondary nucleus.

After fertilization, the endosperm nucleus divides first and gives rise to the nuclear endosperm (Fig. 45). The oospore divides repeatedly until a linear, uniseriate eight-celled proembryo is formed (Fig. 47). The cells towards the embryonal end undergo anticlinial divisions and become biseriate (Figs. 48 & 49). The embryo proper appears to be developed from the three or four apical layers (Figs. 50 & 51). The portion of the suspensor immediately next to the embryo proper is bi-seriate. Multiseriate suspensors occur in other genera of Rubiaceæ, where the cells elongate and perform haustorial functions, for example, *Asperula*, *Vaillantia*, *Galium*, etc.

The mature seed is enclosed in the locule of the ovary whose wall becomes much thickened.

V. Discussion

(*a*) *Some cyto-morphological considerations.*—In a previous paper, Raghavan and Rangaswamy (1941) have discussed the basic chromosome number of the family Rubiaceæ and have suggested 11 as the possible number. In *Oldenlandia* and *Dentella* a rudimentary (single-celled) nucellus was met with. This advanced feature was correlated to the chromosome number being in the nine series. It was therefore suggested that 9 could not be the basic number, but some other number higher than that. The most frequently occurring number was 11 and this was exhibited by members possessing the so-called primitive type of nucellus, *i.e.*, the *Phyllis*-type of nucellus with seven to eight nucellar cells forming a convex layer capping the archesporial cell. The other numbers were suggested to have arisen secondarily by various means.

The present morphological study of these two genera seems to corroborate the previous findings. Some interesting observations suggest themselves on a closer scrutiny of the chromosome numbers known so far, as correlated to this important morphological feature, *viz.*, the nature of the nucellar epidermis. Based chiefly upon this factor, 11 was suggested to be the possible basic number of the family. Members exhibiting this number are characterised by the so-called *Phyllis*-type of primitive nucellar epidermis, composed of 6 or 7 cells forming a convex layer. The other members derived therefrom must be regarded as secondary numbers and as such the genera exhibiting these numbers must show a type of nucellar epidermis which must be regarded as more

advanced than the *Phyllis*-type. *Prima facie* these two genera investigated in this paper, *Spermacoce* and *Guettarda*, corroborate these inferences. *Guettarda* which is in this investigation reported to have $n = 22$ (belonging to the 11 series) must, if the previous suggestion was correct, show the *Phyllis*-type of nucellar epidermis (Fig. 39 A). And this is just what we find. To secure further corroboration for this, we have studied the nucellar epidermis in other genera showing the 11 number. The genera that we have studied and figured are *Ophiorrhiza*, *Canthium* and *Mussaenda* (Figs. 39 B, 39 C & 39 D respectively). These show definitely the *Phyllis*-type of nucellus. *Pentas*, which is another species investigated (Fig. 39 E), shows $n=10$ and in this, the nucellus is not of the *Phyllis*-type, but of the *Bouvardia*-type, *i.e.*, two to four cells forming a straight row.

We have made a critical study of the data presented in Fagerlind's paper and endeavoured to find out, if the evolution of the nucellar epidermis such as is indicated by Fagerlind, could in any way throw light on the evolution of the chromosome numbers in the family. According to Fagerlind, who does not use chromosome numbers to support his views upon nucellar evolution, the *Phyllis*-type is the most primitive. On a close examination of the different genera comprised under this group, the vast majority belong to the 11 series. The only exception would appear to be *Hoffmannia* showing $n = 12$. The genera coming under this group are as follows:—*Phyllis*, *Psychotria*, *Cephalanthus*, *Chiococca* (all belong to the 11 series) and *Hoffmannia* ($n = 12$) (Fagerlind), *Ophiorrhiza*, *Canthium*, *Guettarda* and *Mussaenda* (all belong to 11 series) (Authors).

From this *Phyllis*-type the *Bouvardia*-type is derived, wherein the nucellus is straightened with about four cells. Plants showing this type of nucellus are *Bouvardia* ($n = 9$) and *Pentas* ($n = 10$). From this *Bouvardia*-type, according to Fagerlind, evolution has proceeded along two lines, one resulting in the *Oldenlandia*-type with only one nucellar cell. Along this line reduction has presumably taken place from a four-celled condition to an one-celled condition as in *Oldenlandia* and *Dentella*. A still further reduction along this line leads to the complete suppression of the nucellar cells, *e.g.*, *Houstonia*. Along the other line evolution is suggested to have taken place in the direction of increased nucellar cells culminating in the so-called *Vaillantia*-type. Plants showing this type of nucellus are *Mericarpea* ($n = 11$), *Galium* ($n = 10$ and 11), *Callipeltis* ($n = 11$), *Sherardia* ($n = 11$), *Rubia* ($n = 11$), *Putoria* ($n = 11$), *Warburgina* ($n = 11$), *Asperula* ($n = 10$ and 11), *Phuopsis* ($n = 10$ and 11), *Vaillantia* ($n = 9$), *Spermacoce* ($n = 14$) and *Richardsonia* ($n = 14$) (Fagerlind). Whereas in *Bouvardia*-type there is only a straight row of two to four cells,

there are about six to seven cells in the *Vaillantia*-type. This scheme of nucellar evolution is shown diagrammatically in Diagram A.

Trying to correlate the chromosome numbers known so far, with this line of evolution as suggested on the basis of the nucellar epidermis alone, we find that, though in the main it is corroborative, yet there are a few difficulties. If it is conceded that 11 is the primitive number—for this there seems to be ample evidence now at hand—then, the difficulty would arise in respect of the *Vaillantia*-type. This, according to Fagerlind, possesses an advanced type of nucellus with a straightened row of seven cells, having been derived from the *Bouvardia*-type. If this is so, the majority of the plants belonging to this type must show a number other than 11, either more or less. On the other hand, we find that the majority of the genera under this type would appear to possess 11. *Vaillantia* itself shows only 9. There are others which show numbers higher than 11, *e.g.*, *Spermacoce* and *Richardsonia*. Except *Vaillantia* all the others show either 11 or generally more than 11. It would therefore appear to us more reasonable to derive this *Vaillantia*-type directly from the *Phyllis*-type, without the intervention of the *Bouvardia* stage. Cytologically it would mean the evolution from a eleven-chromosomed *Phyllis* ancestor, of genera showing chromosome numbers of 11 and more, exhibiting the straightened type of nucellus. We venture to believe that the evolution of a straightened seven-celled nucellus from a convex seven-celled nucellus would be more natural, than its evolution from a *Bouvardia*-type. The latter method would mean that, first of all, from a convex seven-celled nucellus the *Bouvardia*-type was derived involving not only a straightening, but also a reduction in the number of cells. This reduced number has to increase again in order to attain the *Vaillantia* condition. This is rather strained and we believe that the evolution of the *Vaillantia*-type directly from *Phyllis* would be more natural. The chromosome numbers also seem to support this view.

On the other side, from the *Phyllis*-type, the *Bouvardia*-type may be derived. By further reduction the *Oldenlandia*-type is got. Still further reduction leads to the *Houstonia*-type. On the basis of chromosome numbers such an evolution would appear to be supported. All these types show chromosome numbers less than 11. *Bouvardia*-type includes genera showing 9 and 10. *Oldenlandia* and *Houstonia*-types include genera belonging to the nine series only. This scheme of evolution is represented diagrammatically in Diagram B.

Evolution presumably has proceeded along two divergent lines from the *Phyllis*-type. Along one line the number of nucellar cells has remained

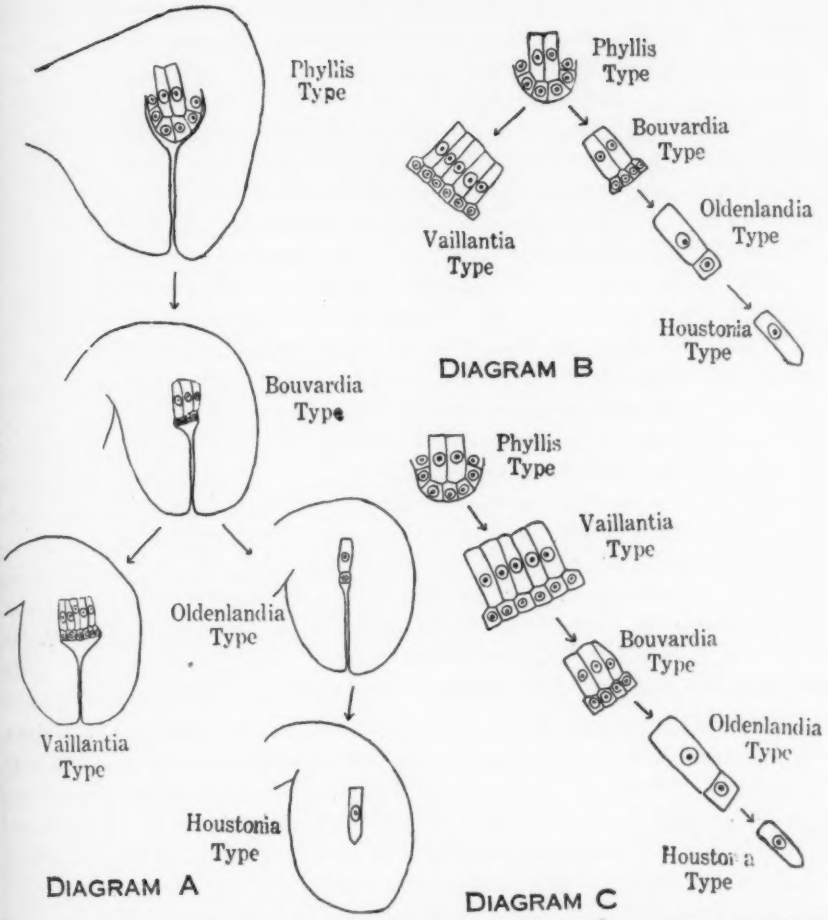


DIAGRAM A. Showing the evolution of the nucellus in the Rubiaceæ according to Fagerlind's concept.

DIAGRAMS B & C. Illustrating alternative schemes of nucellar evolution suggested in the present paper. Explanation in the text.

constant, only they have straightened out. In these, chromosome numbers have also remained unchanged in the vast majority of cases and such of the deviations as are met with like *Spermacoce* and *Richardsonia*, are on the side of increased numbers. Along the other line, nucellar reduction would appear to be correlated with reduction in chromosome numbers, all these belonging to the nine series.

There is yet another possibility that should also be considered, *viz.*, the straight evolution along a single line from the *Phyllis*-type instead of their being two divergent lines of evolution. From the *Phyllis*-type we get the *Vaillantia*-type (straightened, but same number of cells) from *Vaillantia*, the *Bouvardia*-type (straightened, but number of cells reduced), from this the *Oldenlandia*-type (still further reduction to single cell) and ultimately to the *Houstonia*-type (nucellus completely suppressed). This scheme has its merits also. Primarily the stray occurrence of numbers less than eleven in the *Vaillantia*-type (*Vaillantia*—9, *Phuopsis*—10, *Asperula*—10), may be explained, *i.e.*, from the *Phyllis*-type to the *Vaillantia*-type there is no change in the number of nucellar cells, only there is a straightening. The majority of the plants remain unchanged in chromosome numbers also, but, even in this group, we already see a tendency towards a reduction in the chromosome numbers from 11 to 10 and 9. This becomes a constant feature in the succeeding types like *Bouvardia*, *Oldenlandia* and *Houstonia*. Only, in these groups this reduction of chromosome number is accompanied by a reduction in the nucellar epidermis. In *Bouvardia*, with only four cells, we get nine and ten. In *Oldenlandia*- and *Houstonia*-types, we get only nine seried plants so that, the ten and nine plants, the few that there are under the *Vaillantia*-type, must be regarded as representing a tendency for reduced chromosome numbers unaccompanied by a reduction in the nucellar cells. This however soon follows and the result is the *Bouvardia*-type from which the others arise. This is represented schematically in Diagram C.

It must also be admitted at the same time that in the very *Vaillantia*-type, there are some genera showing numbers more than 11, *Spermacoce* and *Richardsonia*. What these portend we do not know, primarily because we have no morphological evidence for plants showing numbers higher than 14, etc., for *e.g.*, for *Posoqueria* belonging to the 17 series we have no information about the nature of nucellus.

We have comparatively enough data for plants exhibiting the numbers less than 11. All these, whether belonging to *Bouvardia*- or the *Oldenlandia*-type, show reduced nucellus either four or one-celled. Their number is most generally 9, ten being shown by very few only. Since these numbers occur occasionally in the *Vaillantia*-type, we have suggested that these reduced numbers, unaccompanied by reduced nucellus, already portend the reduced nucellus, that becomes the constant feature in the *Bouvardia* and *Oldelandia* types. In the same way, what the stray occurrence of increased numbers like 17, etc. are suggestive of, we can only say if we had some data pertaining to the nature of nucellus in plants showing the higher numbers. We know that

the 14-seried plants like *Spermacoce* show the *Vaillantia*-type of nucellus. In other words, just as a stray decrease of chromosome numbers amongst this type unaccompanied by any nucellar reduction, has been held to exhibit the tendency for nucellar reduction, such as is exhibited by the *Bouvardia* and *Oldenlandia* types, so, the increased numbers must be regarded as indicating a tendency towards increased nucellar cells. If we had details for any higher chromosomed genera, then, this anticipation could be verified even as in the case of the reduced numbers correlated to reduced nucellar cells. But, pending this, the suggestion can only be made that the increased numbers must either show the *Vaillantia*-type of nucellus or a nucellus with more cells than in the *Vaillantia*-type. Whether this anticipation is correct, future investigations alone can tell.

(*b*) *Strophiole*; *has it any phylogenetic significance?*—In Rubiaceæ, it is difficult to find a morphological character of strict phylogenetic significance except the nucellar epidermis. The strophiole is present in most of the genera irrespective of whether the genera are primitive or advanced, determined from apt considerations like the nature of nucellus. Thus for example, it is present in *Guettarda* which has a primitive nucellus and is at the same time present in *Spermacoce* which has got an advanced type of nucellus. Moreover, it does not characterise genera which belong to a particular chromosome type. It does not even characterise the genera belonging to tribes of ordinary classification, based on the number of ovules in the carpel. It is thus present both in Coffeoideæ and Cinchonoideæ, though only rarely in the latter. So the presence of strophiole can be dismissed as possessing little phylogenetic significance.

VI. Summary

The development of embryo-sac and embryo has been described in *Spermacoce hispida* and *Guettarda speciosa*.

The haploid numbers, *Spermacoce* ($n = 28$) and *Guettarda* ($n = 22$), previously reported, are confirmed. Meiosis is described in some detail.

The development of the strophiole has been traced and it is considered to have little phylogenetic importance.

The suggestion previously made that 11 was the basic number of the family is confirmed by data gathered in this paper.

The evolution of the chromosome numbers in this family is discussed in the light of data known in connection with nucellar evolution. A correlation is established between the two and a few tentative schemes of nucellar and chromosomal evolution suggested.

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ACTION OF SULPHANILAMIDE DERIVATIVES IN EXPERIMENTAL STREPTOCOCCAL AND PNEUMOCOCCAL INFECTIONS IN MICE: PART II*

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This paper presents the results of our testing thirty-six out of about eighty sulphanilamide derivatives synthesised so far at this Institute[1] in experimental β-hæmolytic streptococcal and pneumococcal (type I) infections in mice. The outstanding therapeutic effects of one of them, 2-N^1-sulphanilamidothiazole (sulphathiazole), in experimental plague,[2] many bacterial and some virus infections[3] have already been described.

The results obtained are also discussed here from the point of view of the relationship of chemical constitution to antibacterial action. For the sake of convenience, in the protocols and discussion, the compounds tested will be referred to by our serial numbers which are given before each compound in brackets.

The following compounds were tested:—

(N^4)-Amino-substituted Sulphanilamide Derivatives:

(11) 4-Sulphonamidobenzenethiocarbamide:
$NH_2 \cdot CS \cdot NH \cdot C_6H_4 \cdot SO_2NH_2$.

(22) N-4-Sulphonamidophenyl-N′-phenylthiocarbamide:
$Ph \cdot NH \cdot CS \cdot NH \cdot C_6H_4 \cdot SO_2NH_2$.

N^1-Sulphanilamidobenzene Derivatives:

(1) 4-(N^1-Sulphanilamido)nitrobenzene:
$NH_2 \cdot C_6H_4 \cdot SO_2NH \cdot C_6H_4 \cdot NO_2(4')$.

(5) 3-(N^1-Sulphanilamido)nitrobenzene:
$NH_2 \cdot C_6H_4 \cdot SO_2NH \cdot C_6H_4 \cdot NO_2(3')$.

(6) 2-(N^1-Sulphanilamido)nitrobenzene:
$NH_2 \cdot C_6H_4 \cdot SO_2NH \cdot C_6H_4 \cdot NO_2(2')$.

(20) 4-(N^1-Sulphanilamido)dimethylaniline:
$NH_2 \cdot C_6H_4 \cdot SO_2NH \cdot C_6H_4 \cdot NMe_2(4')$.

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* Part I of this series: *Indian Jour. Med. Res.*, 1940, **27**, 971.

Azo dyes from Sulphapyridine and Sulphathiazole:

(9) 2-N^1-Sulphanilamidopyridine diazotised and coupled with *m*-phenylenediamine.

(10) 2-N^1-Sulphanilamidopyridine diazotised and coupled with 4-aminothiouracil.

(21) 2-N^1-Sulphanilamidothiazole diazotised and coupled with 4-aminothiouracil.

Pyridine Derivative:

(16) 2-(4′-N^1-Sulphanilamidobenzenesulphonamido)pyridine (Formula I).

(N^4)-*Amino-substituted Quinoline Derivative*:

(7) 2-(4′-Sulphonamidobenzeneamino)quinoline-3-carboxylic acid (Formula II).

(N^1)-*Sulphonamido-substituted Acridine Derivative*:

(8) 2 : 8-Di(N^1-sulphanilamido)acridine.

(N^4)-*Amino-substituted Acridine Derivatives*:

(23) N-(9′-Acridyl)sulphanilamide (Formula III, R = H).

(24) 2-[N^4-(9′-Acridyl)sulphanilamido]pyridine (Formula III, R = 2-pyridyl).

(26) 4-[N^4-(9′-Acridyl)sulphanilamido]aniline (Formula III, R = $-C_6H_4\cdot NH_2$).

(27) 4-[N^4-(9′-Acridyl)sulphanilamido]benzenesulphonamide (Formula III, R = $-C_6H_4\cdot SO_2NH_2$).

(28) 4-[N^4-(9′-Acridyl)sulphanilamido]nitrobenzene (Formula III, R = $-C_6H_4\cdot NO_2$).

N^1-*Sulphonamido-substituted Guanidine Derivatives*:

(56) Sulphanilylguanidine:

$$NH_2\cdot C_6H_4\cdot SO_2N:C\begin{matrix}NH_2\\NH_2\end{matrix}$$

(58) N^4-Sulphanilylsulphanilylguanidine:

$$NH_2\cdot C_6H_4\cdot SO_2NH\cdot C_6H_4\cdot SO_2N:C\begin{matrix}NH_2\\NH_2\end{matrix}$$

N^1)-*Sulphonamido-substituted Thiazole Derivatives*:

(13) 2-N^1-Sulphanilamidothiazole (Formula IV, R = H).

(33) 2-N^1-Sulphanilamido-4-methylthiazole (Formula IV, R = Me).

(45) 2-[4′-N^1-Sulphanilamidobenzenesulphonamido]thiazole (Formula V).

(N^4)-Amino-substituted Thiazole, Thiazolin & Pseudothiohydantoin Derivatives:

(40) 2-(4′-Sulphonamidobenzeneamino)thiazole (Formula VI, $R = R' = H$).

(37) 2-(4′-Sulphonamidobenzeneamino)-4-methylthiazole-5-ethylcarboxylate (Formula VI, $R = Me$; $R' = CO_2Et$).

(39) 2-(4′-Sulphonamidobenzeneamino)thiazole-4-ethylacetate (Formula VI, $R = -CH_2 \cdot CO_2Et$; $R' = H$).

(41) 2-(4′-Sulphonamidobenzeneamino)-4-methylthiazole-5-ethylacetate (Formula VI, $R = Me$; $R' = -CH_2 \cdot CO_2Et$).

(34) 2-(4′-Sulphonamidobenzeneimino)-3-allylthiazolin (Formula VII, ($R = allyl$; $R' = H$).

(38) 2-(4′-Sulphonamidobenzeneimino)-3-phenylthiazolin (Formula VII, $R = Ph$; $R' = H$).

(36) 2-(4′-Sulphonamidobenzeneimino)-3-allyl-4-phenylthiazolin (Formula VII, $R = allyl$; $R' = Ph$).

(46) 2-(4′-Sulphonamidobenzeneamino)-5-iodomethyl-Δ^2-thiazolin (Formula VIII).

(44) N^2-4-Sulphonamidophenylpseudothiohydantoin (Formula IX).

(N^1)-Sulphonamido-substituted Heterocyclic Derivatives:

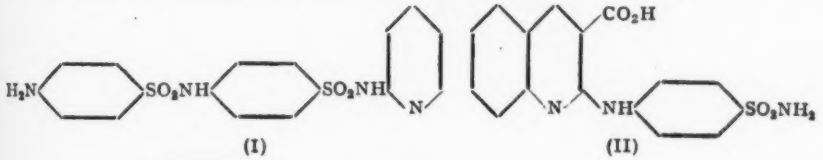
(50) 7-N^1-Sulphanilamidoalloxazine (Formula X).

(51) 2-N^1-Sulphanilamido-5-methyl-1 : 3 : 4-thiodiazole (Formula XI).

(54) 5-N^1-Sulphanilamidobarbituric acid (Formula XII). (5-N^1-Sulphanilamido-2 : 4 : 6-trioxypyrimidine).

(53) 4-N^1-Sulphanilamidouracil (Formula XIII).

(57) 2-N^1-Sulphanilamido-4-methylpyrimidine (Formula XIV).



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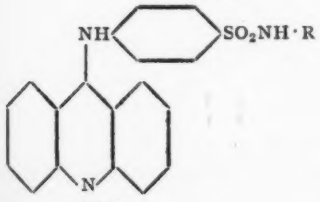

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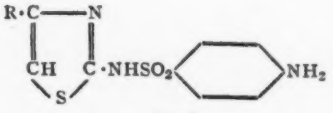

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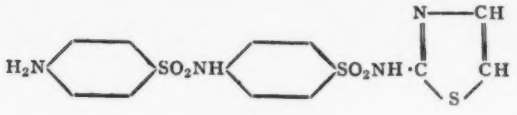

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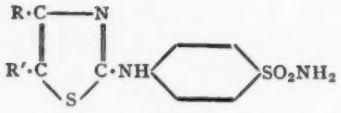

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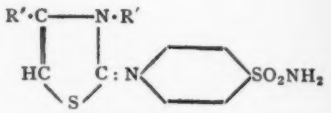

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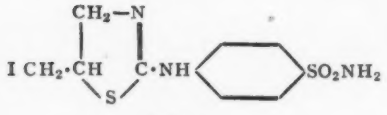

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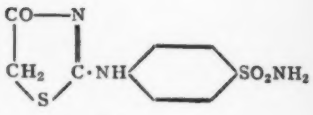

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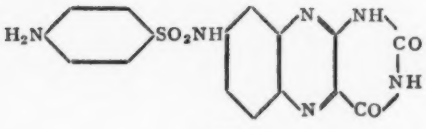

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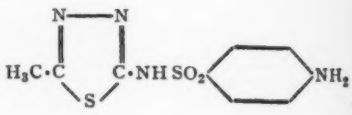

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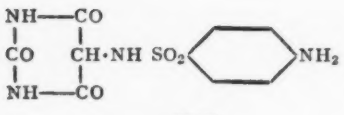
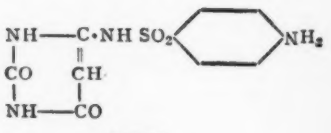
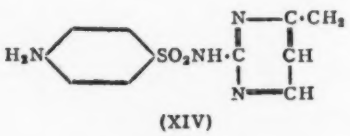


(XII)



(XIII)



(XIV)

Experimental Results

The method of testing has been described in detail in a previous publication. ' Richards ' strain of β-hæmolytic streptococcus was used, the infecting dose being 0·5 c.c. of a 1 : 6 dilution of a 17-hr. old broth culture. In the case of the pneumococcus (type I), this dose was 0·5 c.c. of 1 : 2000 dilution of a 17-hr. old broth culture. The drugs were administered as suspensions (0·5 c.c.) in gum acacia.

The toxicity of each of the drug was tested first, to be sure that the doses administered were not themselves lethal. Many of the inactive ones were tested in doses as high as 50 mg.

The compounds 7, 8, 11, 21, 26, 27, 28, 34, 36, 37, 38, 39, 40, 41, 44, 46, 50, 53, 54 and 58 were very little or not active against both the infections. The results obtained with the other compounds are given below in the tables.

TABLE I

Therapeutic Effect in Experimental Streptococcal Infections in Mice

Experiment No.	Drug administered	No. of mice used	Dosage in mg.	No. of mice dying on each day after infection							Survivors	Average survival time (days: max. 10)
				1	2	3	4	5	6	7–10		
I	The drugs were administered at the time of infection and repeated 6 hours later and then once a day for 3 days more											
	S.A.	6	10	2	1	1	0	0	0	0	2	3·8
	1	6	5	3	0	0	0	0	1	0	2	4·5
	5	6	5	2	0	0	0	0	0	2	2	5·8
	6	6	5	0	0	1	1	0	3	0	1	5·0
	10	6	10	1	0	0	0	0	0	3	2	6·8
	13	6	10	0	0	0	0	0	0	2	4	9·0
	23	6	10	1	1	1	0	1	0	1	1	4·0
	24	6	10	0	1	1	0	0	2	1	1	5·0
	26	6	10	2	2	2	..	..	..	..	0	1·0
	C.	6	..	5	1	..	..	..	..	..	0	0·2
II	The drugs were administered at the time of infection and repeated 6 hours later and then twice daily for 3 days											
	20	6	10	2	1	1	0	0	0	0	2	3·8
	45	6	10	2	1	0	0	0	0	0	3	5·1
	C.	6	..	6	..	..	..	..	..	..	0	0·0
III	The drugs were administered as in Experiment II											
	56	10	20	5	2	0	1	0	0	0	2	2·5
	57	10	10	7	0	1	0	0	0	0	2	2·2
	13	10	10	2	0	1	0	0	0	0	7	7·2
	C.	10	..	8	1	1	..	..	..	..	0	0·3

S.A. = Sulphanilamide.

C. = Controls (no treatment given).

TABLE II

Therapeutic Effect in Experimental Pneumococcal Infections in Mice

Experiment No.	Drug administered	No. of mice used	Dosage in mg.	No. of mice dying on each day after infection					Survivors	Average survival time (days: max. 10)
				1	2	3	4	5–10		
I	The drugs were administered at the time of infection and repeated 6 hours later and then once a day for 5 more days									
	S.P.	10	30	0	0	0	3	0	7	7·9
	13	10	30	0	0	1	1	0	8	8·5
	16	10	30	1	6	3	..	..	0	1·2
	24	10	30	2	5	3	..	..	0	1·0
	C.	10	..	7	3	..	..	..	0	0·3
II	The drugs were administered at the time of infection and repeated 6 hours later and then twice daily for 3 more days									
	20	6	20	3	0	2	0	0	1	2·3
	21	6	20	3	2	1	0	0	0	0·7
	45	6	20	3	2	0	0	1	0	1·0
	C.	6	..	5	1	..	..	..	0	0·2
III	The drugs were administered at the time of infection and repeated 6 hours later and then twice daily for 2 more days									
	33	12	20	0	0	0	3	1	8	7·8
	51	6	20	0	2	1	1	0	2	4·8
	C.	6	..	4	1	1	..	..	0	0·5
IV	The drugs were administered at the time of infection and repeated 6 hours later and then twice daily for 5 more days									
	57	10	5	0	0	0	1	3	6	8·2
	57	10	10	0	0	0	1	3	6	8·4
	13	10	10	3	1	0	0	3	3	4·8
	13	10	20	0	0	0	0	3	7	8·9
	C.	10	..	9	1	..	..	..	0	0·1

S.P. = Sulphapyridine.
C. = Controls (no treatment given).

It can be seen that the drugs 1, 5, 6, 10, 13, 20, 23, 24, 45 and 57 are at least as active as sulphanilamide against the streptococcus infection in the doses tried. But against pneumococcus infection only 13, 33 and 57 gave a very high and 56 and 51 moderate protection. It is very striking that the pyrimidine derivative, 57, in as low a dose as 5 mg. gives almost the same protection as sulphathiazole, 13, in 20 mg. doses. The rest of the compounds were inactive or only slightly prolonged the lives of the infected animals.

Discussion

The synthesis and study of the sulphanilamide derivatives undertaken, though they have for their primary objective the discovery of drugs superior

to sulphanilamide or sulphapyridine (2-sulphanilamidopyridine), also constitute a systematic attempt to explore the fundamental relationship existing between chemotherapeutic activity and molecular structure. The results presented above have revealed many significant facts in this direction.

In a previous publication,[4] we have produced evidence strongly supporting the hypothesis of Tréfouel *et al.*[5] that the therapeutic activity of 'prontosil' is due to sulphanilamide liberated from it *in vivo* by reduction. To investigate this question further, we tested the three azo dyes, 9, 10 and 21, prepared from sulphapyridine and sulphathiazole. It was intended to see whether the dyes are active in pneumococcus infection since they should owe their activity to the two parent drugs produced *in vivo*. While the dye 21 was almost inactive, dye 10 showed considerable antistreptococcal and very little antipneumococcal activity. Though, superficially, this appears to be argument against the hypothesis of Tréfouel *et al.*,[5] a closer scrutiny reveals the implications of these results. In the dosage used in the experiments the dye 10 produced blood concentrations of about 2 mg. per cent. of sulphapyridine and the dye 21 very little sulphathiazole, while in experiments wherein the drugs sulphapyridine and sulphathiazole themselves were employed, their corresponding blood concentrations were of the order of 10–25 mg. per cent. (unpublished observation). It has been shown by comprehensive experiments by Marshall that a blood concentration of 2–3 mg. per cent. of the above two drugs can give good protection against streptococcal infection, while only 7–9 mg. per cent. of it can do so in pneumococcal infection. Thus the good protection in streptococcal infection and very little protection in pneumococcal infection of the dye 10 and the very feeble activity of the dye 24 are just these to be expected according to the hypothesis of Tréfouel *et al.* It is because of the low blood concentration of sulphanilamide produced following the administration of 'prontosil' that this drug is not at all recommended in the treatment of severe infections. As has been pointed out by us previously,[4] a strong case has yet to be made to recommend the 'prontosils' or other similar azo dyes in preference to sulphanilamide for clinical trials.

A major number of the compounds which possess substituents at the (N^4)-amino radical of sulphanilamide or (N^1)-sulphanilamido derivatives, are all inactive probably because they do not undergo fission at the amino linkage to produce *in vivo* sulphanilamide or sulphanilamido derivatives. The inactive compounds, 11, 22; 7; 26, 27, 28; 34, 36, 37, 38, 39, 40, 41, 44, 46 come under this category. Two of the acridine derivatives, 23 and 24, reported are moderately active in streptococcal and very little active in pneumococcal infections. The activity of these two drugs is quite possibly

due to the liberation from them of the free sulphanilamide and sulphapyridine respectively *in vivo*, since such 9-substituted acridine derivatives are well known to undergo fission at this position. Since the production of adequate blood concentration of the sulphanilamido derivatives from these acridine compounds depends upon the rate of fission mentioned above as well as the degree of absorption in the system of the original acridine complex and since these factors naturally do vary with each compound, we find that of this class of compounds some are active while others are not. The therapeutic activity of the compounds 23 and 24 thus resembles that of N^4-benzylsulphanilamide (' proseptazine ') which owes its activity to sulphanilamide liberated from it *in vivo*.[7] A comparison of the two isomeric thiazole derivatives, sulphathiazole, 13, which is remarkably active and the compound 40 which is absolutely inactive—the difference between the two being the thiazole ring is substituted in the former at the (N^1)-sulphonamide radical and in the latter at the (N^4)-amino radical of sulphanilamide—clearly shows the importance of a free amino group as also the substitution of the specified heterocyclic ring at the sulphonamido group for the production of pronounced therapeutic activity.

Regarding all the compounds considered hitherto which form one group, wherein substituents anticipated to enhance the therapeutic property are introduced at the (N^4)-amino radical of sulphanilamide or sulphanilamido derivatives, we can conclude as follows: the therapeutic activity of these compounds appears to depend upon whether or not they can undergo fission at the (N^4)-amino linkage to yield free sulphanilamide or sulphanilamido derivatives *in vivo* and the degree of therapeutic effect depends upon the effective blood level of the latter produced *in vivo*. This conclusion just fits in with the various theories of the mechanism of action of the sulphanilamides that assign some important biochemical or physico-chemical functions to the free (N^4) amino group in the production of the therapeutic activity. The compounds of this class, whenever active—*e.g.*, ' prontosils ', ' proseptazine ', ' soluseptazine ', the Schiff's bases, etc.,—are less polyvalent in therapeutic action than the parent drugs, evidently because the effective blood levels of the active compounds produced *in vivo* following their administration are so low as to control only mild and not severe infections.[6]

Of the many aliphatic, aromatic and carbocyclic substituents tried at the sulphonamide radical of sulphanilamide, only the benzene derivatives have not decreased the therapeutic activity of sulphanilamide. The three isomeric nitro derivatives, 1, 5 and 6, of N^1-sulphanilamidobenzene were tested in view of the therapeutic significance attached to the nitro group by Rosenthal *et al.*[8] Though these compounds were somewhat more active

than sulphanilamide in streptococcal infection, they were more toxic. Their antipneumococcal effect compared to sulphapyridine or sulphathiazole was negligible. The compound 20 also possessed considerable anti-streptococcal but very little antipneumococcal effect. Taking all the N^1-sulphanilamidobenzene derivatives tested hitherto as a whole, it can now be generalised that though many derivatives are available somewhat superior to sulphanilamide in antistreptococcal effect, they possess no distinct advantages to replace sulphanilamide in practical therapy; further, they are nowhere near in therapeutic effect to sulphapyridine or sulphathiazole in pneumococcal infection.

N^4-sulphanilylsulphanilamide is reported by many groups of workers to be about as active as sulphanilamide. But the corresponding sulphanilyl derivatives of sulphapyridine 16 (formula I) and of sulphathiazole, 45 (formula V) are distinctly less active than the parent compounds. This appears to indicate that the molecular sizes of sulphapyridine and sulphathiazole appear to be the optimum for maximum therapeutic activity. While sulfanilylguanidine, 56, shows some distinct therapeutic activity, the similar sulfanilyl derivative of this compound, 58, is inactive. These instances indicate that the mere multiplication of the sulfanilamido groups in a compound does not help in obtaining mere potent compounds.

The conclusions drawn above together with the fact that sulphapyridine is more polyvalent in therapeutic effect than sulphanilamide, urged us to synthesise and test sulphanilamido derivatives with heterocyclic rings substituted in the sulphonamide radical. The thiazole derivative, 13, thus obtained has proved to be of outstanding value as has been previously reported.[1, 2, 3] We hope to test a series of related derivatives of this compound after our preliminary survey of the different rings is over. The thiodiazole derivative, 51 (formula XI), which very closely resembles the thiazole derivatives, 13 and 33 (formula IV), in molecular structure, appeared to be far less active than the latter in our preliminary experiments. The fact that pyridine and thiazole rings are present in some vitamins and coenzymes, led us to try the alloxazine derivative, 50, this ring system being present in the vitamin, riboflavin (which forms part of Warburg's "yellow enzyme" and also the coenzyme of amino oxidase); but this compound was found to be absolutely inactive. Some derivatives of the pyrimidine group (this ring system being present in vitamin B_1 connected to a thiazole derivative) have already been synthesised and tested. 5-Sulphanilamidobarbituric acid, 54, and 4-N^1-sulphanilamidouracil, 53, have been found to be inactive, while 2-N^1-sulphanilamido-4-methylpyrimidine, 57, is remarkably active. These agree with the results reported by Roblin *et al.*[9] A very detailed study

is being made with the 2-N^1-sulphanilamidopyrimidine derivatives synthesised here and these will be reported in a later communication.

We thank Lt.-Col. S. S. Sokhey, M.D., I.M.S., Director, for his interest in this work and also the Lady Tata Memorial Trust for the award of a scholarship to one of us (K. G.).

Summary

The results of testing thirty-six derivatives of sulphanilamide of various groups in experimental β-hæmolytic streptococcal and pneumococcal (type I) infections in mice have been presented. The therapeutic activity of the derivatives with substituents in the (N^4)-amino radical of sulphanilamide or sulphanilamido derivatives, appears to depend upon whether or not they can yield *in vivo* the latter compounds in adequate concentrations. In the attempts to discover highly active compounds, the study of the derivatives of sulphanilamide with suitable heterocyclic rings substituted at the sulphonamide radical have been made. Of the many such compounds so far tested, the sulphanilamidothiazole derivatives, 13 and 33, and the sulphanilamidopyrimidine derivative, 57, have been found to be of outstanding value. The significant relationship existing between the chemical constitution and chemotherapeutic action has been pointed out.

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ERRATA

In the title of the paper, page 437, *add* footnote mark " † " to " *Pempheres affinis* " and *insert* the following footnote:—

† *Pempheres affinis* is now known as *Pempherulus affinis* (*Ann. Mag. Nat. Hist.*, 1941, 7).

Page 439, in the list of species *transfer* the word " *Chalcidoidea* " to the top of the species in both the columns.

Page 443, last line of the para *General biology—for* " stages " *read* " stage ".

THE BIOLOGY AND DISTRIBUTION OF THE PARASITES OF THE COTTON STEM WEEVIL, *PEMPHERES AFFINIS* FST. IN SOUTH INDIA*

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(Communicated by Rao Bahadur V. Ramanatha Ayyar)

Introduction

For nearly thirty years *Pempheres affinis* Fst. has been accounted as one of the major pests of cottons grown in S. India. The weevil seems to be a highly adaptable species occurring over a wide range of conditions. Though the distribution of the insect as a pest is confined to the chief cotton-growing districts of S. India, recent studies have revealed that it is a normal inhabitant of most non-cotton areas in India. Great injury is caused by the extensive tunnelling of the stem by the immature stages which results in considerable losses. Heavy mortality of plants (20–25 per cent.) occurs especially in the early stages.

The infestation commences fairly early in the season by October when the crop is about two weeks old. From a small population of a few immigrants in the initial stages, huge populations are built up causing cent. per cent. attack towards the close of the season in March. Three successive, though somewhat overlapping, generations are undergone by the pest in the cotton season.

Being an internal feeder during the major portion of its life, ordinary methods have been of little avail in its control. With a view, therefore, of furthering its control, the Indian Central Cotton Committee initiated, towards the close of 1935, a small scheme of special studies on the incidence and distribution of the weevil in cotton and other food plants in conjunction with a search for its natural enemies. The results of the studies on several aspects have been embodied in a series of papers by Krishna Ayyar (1936–40). It is the purpose of the present paper to give a connected account of the parasites of this serious pest with particular reference to cotton field conditions.

* Paper read at the Second Conference of Scientific Research Workers on Cotton in India, 1941.

Origin of the Pest

This aspect has a vital bearing on the parasite complex. All available evidence gathered so far converge to suggest that an originally harmless native insect feeding and breeding in wild Tiliaceous and Malvaceous plants (most probably in the species *Triumfetta rhomboidea*) has learnt in recent years to attack an economic plant like cotton. Over thirty years ago *Pempheres* began to attack Cambodia cotton at first only occasionally, later much more persistently, accompanied by an ever-widening distribution. Gradually it acquired a taste for country cottons. While confined to its wild plant it was probably held in check by a complex of ecological factors particularly scarcity or unsuitability of food plants in certain seasons and the parasitic forces. When it transferred its attention to cotton these restraints were eluded. Cotton fields supplied an abundance of food and the natural enemies failed to move into cotton fields. The causes of this failure may be due either to lack of opportunities or their incapacity to attack in the new situation of the weevil's cultivated habitat. The former appears to be the more probable reason.

Previous Records of Parasitism

Prior to the inception of the present studies, no parasites were known to attack *Pempheres* either in India or elsewhere. Ballard (1922) has referred to a chalcid larva in association with *Pempheres*. Soon after the commencement of the Scheme, Ramakrishna Ayyar and Margabandhu (1936) made a brief reference to a few undetermined Chalcids and Braconids noted in association with the weevil in cotton. Later Ramakrishna Ayyar and Mani (1937) named one of these chalcids as *Euderus pempheriphila*. Beyond these brief references nothing was known of the identity of the species concerned, their biology or their importance or true relations to the host. Through the courtesy of Sir Guy A. K. Marshall most of the parasites collected have been named by the specialists of the British Museum. All the species are new records on the host. The great majority of them are new to Science.

Pempheres Parasites

It was impossible within the limited time to make a thorough survey of the parasites or their relative efficiency. The following fifteen species have been bred from the grubs of *Pempheres* in the course of these studies. The exact status of two species (*viz.*, *Eurytoma* sp. and *Aximopsis* sp.) is only partially known. *Pempheres* parasites naturally group themselves into two distinct series since they have been encountered in two different sets of plant associations. A set of species has been recovered from cotton fields, its new habitat of cultivated regions. The others have been exclusively

associated with its alternate food plants, *i.e.*, its old or pre-economic habitat. Among these there are four species which are common to both situations as may be evident from the list furnished below:—

Cotton field parasites *Chalcidoidea*	Alternate food plant parasites *Chalcidoidea*
1. *Euderus pempheriphila* Ayyar & Mani	8. *Entedon pempheridis* Ferr.
2. *Aplastomorpha calandræ* How.	9. *Dinarmus coimbatorensis* Ferr.
3. *Eupelmus urozonus* Dalm.	10. *Bruchocida orientalis* Crawf. *Aplastomorpha calandræ* How. *Euderus pempheriphila* Ayyar & Mani *Eupelmus urozonus* Dalm.
4. *Eupelmella pedatoria* Ferr.	11. *Eurytoma* sp.
5. *Eupelmus* sp.	12. *Aximopsis* sp.
Braconidæ	*Braconidæ*
6. *Spathius critolaus* Nixon	13. *Spathius labadcus* Nixon *Spathius critolaus* Nixon
7. *Microbracon* n.sp.	14. *Rhaconotus cleanthes* Nixon 15. *Rhaconotus menippus* Nixon

Some Common Characteristics of Parasites

All parasites are larval without exception. They are all primary in character. Only one primary (*Eupelmella*) occasionally acts as secondary. *Entedon* is the only endophagous species in the lot. All others are ectophagous. All select only covered hosts as differentiated from those living openly or exposed. The latter habit seems to confer two advantages to the parasites. The covering of the host precludes it from making violent movements which might injure the parasite while it stings the host. Secondly, the inert covering protects it from the attack of hyperparasites. Superparasitism occurs, though infrequently, in most species. Multi-parasitism has not been observed so far.

Extent of Natural Parasitism

The general rate of total parasitism in the cotton field has been comparatively low (*vide* table). The maximum recorded for three years in the seasonal crops and that for off-seasonal crops being 5·20 per cent. (March 1938) and 6·7 per cent. (August 1937) respectively. The extent of parasitism in mortality plants rises a little higher about 13·9 per cent. (March 1936). On the basis of their incidence none can be considered as efficient. Though their incidence is somewhat erratic and irregular, the general trend seems to take a fairly well-defined course as the season advances. In the first generation of the pest (*i.e.*, November–December) the percentage is very insignificant, the maximum being less than 1 per cent. With the second

wave of weevil incidence, the parasitism is more pronounced and the percentage rises in keeping with the increase in host density; unfortunately these favourable conditions do not persist for any length of time. As the parasite forces gain momentum towards the close of the season, the host density tends to decline by the action of a complex of factors such as gumming, proliferation, age of the crop and probably also climatic changes.

The parasites are dealt with individually with special emphasis on those features which would facilitate their recognition in the field. In some cases the chitinized mouth parts and tentorium of the larvæ afford the most reliable distinguishing characters. These can be easily observed in living larvæ under a binocular.

Parasitism

Seasonal Crops				Off-seasonal Crops		
Month	1935–36	1936–37	1937–38	Month	1937	1938
	% parasitism	% parasitism	% parasitism		% parasitism	% parasitism
October ..	Nil	Nil	Nil	May ..	0·26	0·70
November ..	Nil	Nil	1·03	June ..	0·70	0·50
December ..	Nil	Nil	Nil	July ..	3·60	1·40
January ..	0·96	0·90	Nil	August ..	6·70	0·75
February ..	0·20	0·68	Nil	September ..	Nil	3·20
March ..	4·70	2·00	0·54			
April ..	1·50	0·60	5·20			

Mortality Plants—Parasitism

Month	% parasitism		Month	% parasitism	
November 1935 ..	6·65		November 1936 ..	0·92	
December „ ..	Nil	Crops grown in September 1935	December „ ..	0·60	Crops grown in September 1936
January 1936 ..	4·4		January 1937 ..	2·6	
February „ ..	4·2				
March „ ..	13·9				

Cotton Field Parasites

Euderus pempheriphila Ayyar and Mani (Eulophidæ) (Fig. 1, Plate XXI)

This species is by far the commonest and most numerous among the cotton field parasites of *Pempheres* in S. India. The adult is a slender dark insect with a metallic greenish-blue sheen over its entire sculptured

body. The sizes particularly of the female vary widely and fall into two distinct ranges, namely, length: (1·10–1·52 mm. and 1·80–2·21 mm.); males, length: (1·0–1·3 mm., width 0·30–0·42 mm.).

Distribution and host records.—The genus *Euderus* is very widely distributed both in the new and old worlds particularly since the amalgamation of the genera *Secodella* and *Allomphale* as synonyms of *Euderus*. *E. malayansis* Ferr. parasitic on *Prays endocarpa* Meyr. is the first species known from S. Asia (1930). Other species recorded in India are *E. gossypii* Ferr. parasitic on *Sphenoptera gossypii* and *E. lividus* Ashmead on *Agromyza obtusa*. *E. pempheriphila* has been taken from *Pempheres* in cotton in Coimbatore, Erode and Ramnad district as also from the same host in *Triumfetta rhomboidea*, *Sida acuta* in Coimbatore, Thondamuthur, Shemmedu and Malabar. *Hypolixus truncatulus* in *Amaranthus* forms another host for the species.

General biology.—The species has always been found as a primary ectophagous larval parasite. The adults are not strong fliers. They take only short flights in tubes and jars, while handling, displaying phototropic responses. It has not, so far, been amenable to breeding in captivity. All stages from egg to adult have been taken from the fields. In many cases the adults are unable to emerge from the stems and have been taken dead *in situ*. Its efficiency thereby is greatly reduced. A maximum of four parasites has been recovered from a single plant. Parasitism of the species was mostly met with in host stages just underneath the bark. Apparently the female is unable to reach deeper into the stem on account of its small size and minute ovipositor. The stages parasitised in nearly 90 per cent. of the cases were medium sized host grubs. Early and mature grubs are only occasionally attacked. Other stages such as eggs, prepupæ or pupæ are totally rejected. The host is always stung to a state of complete paralysis. Generally only one egg is laid loosely and indiscriminately per host; superparasitism occurs occasionally and two eggs or larvæ may be found on the same host. Three instances of two larvæ on same host have been noted during this investigation and in all these cases none developed probably due to competition and fight. Generally only one adult develops to maturity on a single host. The young whitish larva on hatching feeds externally on body fluids and grows rapidly passing through three instars reducing the host to an empty cuticle and head capsule before turning into a prepupa. The larva has the peculiar habit of standing on its head attached to the host by the mouth parts with abdomen raised. The full grown larva is distinguished by the nature of its mouth parts and strong triangular mandibles. It crawls away from the host-remains and evacuates the meconium in small brownish pellets, and turns to a short white prepupa in the tunnel.

In a day or two it turns into a narrow naked pupa. The pupa is white at first but soon turns dark when the adult emerges.

Immature Stages—Description

Egg (Fig. 2, Plate XXI).—The egg is oblong-ovate in shape, smooth and greyish-white in colour. It is slightly curved and broader at the cephalic end. It measures 0·39 mm. in length by 0·09 mm. in maximum breadth.

I Stage larva.—It is somewhat fusiform in shape with a head and thirteen indistinct segments and measures about 0·40–0·45 mm. in length by 0·10 mm. in width, the breadth of head being 0·07 mm. The spherical blunt head is slightly sclerotized and bears a pair of antennal rudiments. It is whitish except for the dirty brown colour of the stomach. The second stage larva is very similar to this except in larger size and more distended abdomen.

Mature larva.—This stage measures 1·50 mm. in length by 0·55 mm. in width, the head breadth being 0·27 mm. The larva is still whitish with the major portion of the body dirty yellow due to stomach contents.

Prepupa.—The body is short and ivory white roughly demarcated into two distinct regions, the first half of head and thorax and an abdomen narrow at caudal end.

Pupa.—It is flattened in form. At first it is yellowish-white but soon changes to grey-black and later into jet-black. The parts of the adult body are well differentiated.

Seasonal incidence.—The parasite occurs rather late in the cotton season. It puts in its first appearance in January, reaches its peak of abundance in February and continues till the end of March in the seasonal crop. It is generally absent in the first generation of the pest (October–December). It occurs in larger numbers from May–September in the off-seasonal crops with a peak either in June (as in 1937) or July (as in 1938). In Ramnad District its period of occurrence is from July–December with the peak of abundance in September. It may be generalised that it occurs in the second generation of the pest when host density is at the highest.

Life cycle.—Total period 12–18 days, averaging 14 days; Egg period 1 day; Larval period 4–7 days, averaging 5·6 days; Prepupal period 1–2 days, averaging 1·5 days; Pupal period 5–11 days, averaging 7·4 days; Maximum longevity of adults 34 days, averaging 10·1 days.

Natural enemies.—The species is occasionally subject to the attack of hyperparasites. Out of a large number of cases of parasitism observed, only

two instances of attack by hyperparasites have been noticed. The mature larvæ and pupæ are the stages attacked. The hyperparasite concerned is *Eupelmella pedatoria* Ferr.

Aplastomorpha calandræ How.* (Pteromalidæ)

This species is a primary parasite of the grubs of *Pempheres*. It stands second in numerical strength and importance. The adult (Fig. 1, Plate XXII) is a small thickest polished dark insect with a tinge of metallic green. It measures 2·75–3·20 mm. in length by 0·65–0·85 mm. in breadth. The male is much smaller and bears a whitish patch on the abdomen.

Distribution and host records.—It has been collected from Coimbatore, Ramnad and Malabar. It is probably widely distributed in S. India. It is of greater importance as a parasite of various beetles in stored products such as Calandra, Sitodrepa, Lasioderma, Pachymerus and Bruchus. It has been recorded as a parasite of Sitodrepa in stored coriander in S. India. It has been noted during the present studies in Coimbatore to attack Bruchids in Bengal gram, *Bruchus theobromæ* in Phillipasuru, Calandra infesting cholam. This happens to be the first record of its attack on stem-boring weevils. It parasitizes *Pempheres* in a variety of plants such as cotton, *Althæa rosea*, *T. rhomboidea* and *S. acuta*. Another weevil host of the species is *Hypolixus truncatulus* in *Amaranthus* stems.

General biology.—The adult is an active insect capable of sustained flight. Natural light tends to stimulate photographic reactions. The species has a partiality for medium sized grubs of *Pempheres* although mature grubs are almost equally attractive. Early grubs are not totally rejected. In a single instance an egg was seen laid on a prepupa too. Ordinarily prepupæ and pupæ are not accepted. Successful mating and oviposition have been secured in captivity. It reproduces also parthenogenetically giving rise to male progeny. The female does not interest herself in the hosts for two days after emergence. Generally one egg is laid externally per host after stinging the host to quiescence. Occasionally two or more eggs up to a maximum of five are laid. Though more eggs are laid, more than one larva rarely develops to maturity. During development the larva passes through four stages without much morphological change and consumes the entire host contents. The voidance of the meconium in brownish pellets marks its entry into prepupal stages.

* This is probably the same species as *Neocatolaccus indicus* described by Ayyar and Mani, 1937.

Immature Stages—Description

Egg.—The egg is elongate-ovate, translucent and shining white. It is widest in the middle with extremities narrow but the shape varies considerably. The smooth surface appears rough under magnification. It measures from 0·56–0·62 mm. in length by 0·19 mm. in width.

I Stage larva.—Early larva is creamy white with alimentary tract dirty brown due to ingested food. Body is slightly arched and distinctly segmented. It measures 1·00–1·30 mm. in length by 0·25–0·32 mm. in width, the head being 0·19 mm.

Mature larva.—It is spindle-shaped, arched and typically chalcid in form with extremities narrow. It is dirty grey in colour mid-intestine brownish with contents. The head which is fairly conspicuous is roughly semicircular in outline measures 0·41 mm. in width and bears a pair of rod-shaped antennæ. The mouth parts are distinctly ventral. The mandibles are relatively large, heavily chitinized with tips pointed and pigmented. It measures from 2·2–3·4 mm. in length by 0·75–1·20 mm. in breadth.

Prepupa.—It is short greyish-white with a narrow anterior half followed by a wider abdomen.

Pupa.—It is short, stout and naked. The eyes are pinkish-white when newly formed but pigmentation appears the next day. It turns gradually pinkish-brown, deep-brown and later dark in head and thorax and finally dark throughout including legs and antennæ.

Life cycle.—Total period varied widely from 10 (April)–17 days (October); Pre-oviposition period 2–3 days; Egg period 24–30 hours; Larval period 3–6 days, averaging 4–5 days; Prepupal period 15 hours to 3 days, averaging 1·5 days; Pupal period 4–10 days; Maximum adult longevity 40 days, averaging 28 days with raisin as food.

Seasonal history.—It occurs from January–March in the seasonal crops with its peak in March. In Ramnad District it is found in September–October. In off-seasonal crops in Coimbatore it appears in June, steadily increases till July with a decline till September.

Eupelmella pedatoria Ferr. (Eupelmidæ) (Fig. 3, Plate XXIV)

This species is an ectophagous larval parasite of the weevil. This has been encountered only in small numbers in the field and hence it has not been possible to assess its importance in the control of the weevil. The adult female is a small dark insect having greenish and purplish reflections with an average length of 2·6 mm. and witdh of 0·6 mm. It possesses only rudimentary wings. A detailed account of its biology and habits has been

already published (Krishna Ayyar, 1940). Therefore only a brief summary of the most interesting features in its life-history with any additional information since gathered, is presented here.

Distribution and host records.—This is perhaps the first record of the genus in India and the species is new to Science. Its distribution is limited to Coimbatore and surroundings. Other hosts parasitised by the species are the eggs and grubs of *Hypolixus truncatulus* in *Amaranthus* and *Apion corchori* in *Corchorus trilocularis*. It occasionally functions as a hyperparasite on *Euderus pempheriphila*.

General biology.—Males are unknown in the species and reproduction is always by thelyotokous parthenogenesis. Generally one egg is laid per host after partial paralysis by stinging. The larva passes through four stages and completely reduces the host to an empty cuticle before entering the prepupal stage.

Life cycle.—Total period 17–24 days; Preoviposition period 7 days; Egg period 1–2 days; Larval period 5–9 days; Prepupal period 1–3 days; Pupal period 7–12 days; Maximum adult longevity 47 days.

Seasonal history.—The parasite occurs in very small numbers in the seasonal crop during January and February and July–September in the off-seasonal crops. These appear to be more numerous in the latter season.

Eupelmus urozonus Dalm. (Eupelmidæ) (Fig. 1, Plate XXIII)

This species is an occasional parasite of *Pempheres* and, therefore, of only minor importance at present.

Distribution and host records.—This common Palæarctic species has been noted to occur only as far as Egypt and Asiatic Turkey. This constitutes the first record of its occurrence in India. The adult is a dark bluish insect measuring 2·8–3·1 mm. in length by 0·6–0·75 mm. in breadth. It is considered to be an omnivorous species which has been recorded from a wide range of hosts including thirteen species of Hymenoptera, six species of Lepidoptera and six species of Coleoptera. Its coleopterous hosts include curculionids, *Apion pubescens* Kirby, *A. salicis* Gyll, *Cassida filaginis* Perr. (Cassididæ), *Hylesinus vestitus* Muls and Rey, *Scolytus amygdoli* Guer. (Scolytidæ), and *Myelophilus piniperda* L. (pine bark beetles). It has been recorded as playing the rôle of a secondary on *Eurytoma* and *Microgaster*.

It has been reared from *Pempheres* in cotton in Coimbatore, in *Sida acuta* in Malabar and Dhoomanur, *Hibiscus esculentus* in Trichy District. Another weevil host is *Hypolixus truncatulus* in *Amaranthus*.

General biology.—It is a primary ectophagous parasite attacking full grown grubs of *Pempheres*. Occasionally it attacks also medium-sized grubs. Parthenogenetic reproduction is common producing only males. Generally 1–4 eggs are laid on a host after partial or complete paralysis of the host. Often the female after stinging turns round and applies its mouth to the puncture for feeding. The larva passes through four stages before turning to a prepupa.

Immature Stages—Description

Egg.—It is white ellipsoidal and smooth with a pedicel at cephalic end and a small flagellum at the other—approximately measuring 0·51 mm. in length and 0·21 mm. in width.

Mature larva.—It is greyish-white and typically chalcid in form measuring roughly 3·1 mm. in length by 1·0 mm. in breadth.

Pupa.—It is yellowish-white when newly formed, later turns brown and finally dark.

Life cycle.—Total period 14–17 days; Preoviposition period 3 days; Egg period 1–1½ days, averaging 33 hours; Larval period 4–8 days, averaging 6 days; Prepupal period 1 day; Pupal period 6–9 days, averaging 7 days; Adult longevity 18 days.

Seasonal history.—This species has been noted during January–March in the seasonal crops and June–August in off-seasonal crops. On the other host Hypolixus it occurs from November–June.

Eupelmus sp. (Eupelmidæ)

This a primary ectophagous parasite which occurs only occasionally in insignificant numbers. Sufficient material, therefore, could not be obtained for detailed studies. The adult is a small dark insect with greenish reflections. A few specimens submitted to Washington Bureau of Entomology have been named as *Eupelmus* sp.

Distribution and host records.—It has been taken from Ramnad District and Coimbatore. It is rather very rare in the latter place. No host other than *Pempheres* is definitely known. *Hypolixus truncatulus* in *Amaranthus* is suspected to be another probable host.

General biology.—The species generally attacks early stage host grubs and at times medium grubs. The attacks occur only late in the season at the time of multiple infestations by *Pempheres* in the upper portions of cotton stems. Only one parasite develops to maturity per host. The mature larva is dirty white in colour.

Life cycle.—Egg period not known; Larval period about 5 days; Prepupal period 1 day; Pupal period 5–9 days, averaging 6–7 days.

Spathius critolaus Nixon (Braconidæ) (Fig. 2, Plate XXIII)

Spathius critolaus perhaps the most important parasite of *Pempheres* in the cotton field is the only species that occurs during the first generation of the pest. It is capable of attacking the pest in all its diverse plant association. It is comparatively more efficient because of its ability to parasitise stages lodged deep in the stem. It is amenable to mass breeding on other easily available non-economic hosts. It paralyses more stages than actually oviposited upon. It is a wingless reddish-brown ant-like insect which is primary and ectophagous in character attacking mature and medium grubs of *Pempheres*. This species has been fully dealt with in a separate paper (Krishna Ayyar, 1940) and therefore only a few salient points will be recounted briefly here.

Distribution and host records.—This species is probably widely distributed in S. India. It is quite possible that it occurs elsewhere in India. It has been taken from Coimbatore, Erode, Ramnad, Trichy and Malabar. It has been recorded from the Punjab (host not known). It attacks *Pempheres* in a variety of plants such as cotton, *Hibiscus esculentus*, *Triumfetta rhomboidea*, *Sida acuta*, *Corchorus olitorius*, *Pavonia zeylonica*, *Malvastrum*, *H. ficulneus* and probably *Melochia corchorifera*. Other hosts are *Sinoxylon sudanicum* in cotton, *Hypolixus truncatulus* in *Amaranthus* and *Sphenoptera arachides* in Daincha.

General biology.—Generally one egg is laid on one host rarely more up to a maximum of seven, after complete paralysis of the grub. The larva passes through four instars and consumes the entire host contents before starting cocooning in the host tunnel. The adult gnaws its way out of the stem. The developmental stages have been fully described elsewhere.

Life cycle.—Total period 12–27 days according to season; Preoviposition period 2 days; Egg period 1–2 days, averaging 1·2 days; Larval period 3–8 days, averaging 4·7 days; Prepupal period 1–3 days, averaging 2·3 days; Pupal period 5–13 days, averaging 8·6 days; Maximum adult longevity nearly 5½ months on sugar solution and nectar of cotton flowers.

Seasonal history.—It occurs from October–March in seasonal crops and from July–September in off-seasonal crops in Coimbatore. In Ramnad District it has been taken from September–December. It can be reared in laboratory and outdoor cages in all seasons throughout the year on its important alternate hosts and over 9,000 parasites have been thus bred within

a period of over two years (1936–38). Given an abundance of host material, the species can be multiplied in considerable proportions.

Microbracon n.sp. (Fig. 1, Plate XXIII)

This is rather a rare parasite and has been taken actually parasitising *Pempheres* only on two or three occasions. The species has not been definitely identified. The specimens were submitted to the Imperial Institute of Entomology, London, and have been tentatively identified as *Microbracon* n.sp. or Genus nov.

Distribution and host records.—Besides Coimbatore it probably occurs also in Ramnad District. No host other than *Pempheres* is known.

General biology.—This is a primary, ectophagous larval parasite. The adult is a small yellowish wasp measuring 3·1–3·3 mm. in length by 0·9–1·2 mm. in width. Probably only one egg is laid per host as only one larva has been so far noted per host. Medium and mature grubs are accepted as hosts. The host grub is fully consumed before it gets full grown and begins cocooning. On account of its comparative rarity, sufficient material was not available for detailed studies.

Immature Stages—

Mature larva.—The full grown larva is yellowish-white and is in outline elongate-elliptical. It is crescent-shaped and slightly narrower anteriorly than posteriorly. The head and thirteen body segments are well defined. The head is smooth and well chitinized. It approximately measures about 2·5 mm. in length.

Cocoon.—It is constructed of a thin whitish material and is light brownish in colour.

Life cycle.—Pupal period 5 days; Adult longevity maximum 14 days with sugar solution.

Seasonal history.—The species was taken from the field as a mature larva feeding on a full grown host grub on 21st May 1938 for the first time. Again it was recovered as a pupa by the middle of June. On one or two other occasions during the period this species has been observed.

Unidentified parasites

A Chalcidoid having a close resemblance to Eurytoma and a braconid of the genus *Rhaconotus* have been encountered parasitising the grubs in the fields.

Alternate food plant parasites

The parasites listed under this category were totally unknown prior to the present studies. In fact the existence of true alternate food plants for the weevil was itself open to question. These parasites are of extreme importance on account of their occurrence in the weevil's pre-economic habitat. It is an axiom in biological control techinque that the most efficient parasites of an indigenous insect pest have to be looked for in its primitive habitat. Further, these species have an added importance because of their entire absence in the weevil's cultivated habitat, *i.e.*, cotton fields. Most of these parasites are new to Science. These have been dealt with in sufficient detail in a previous publication (Krishna Ayyar, 1940). Hence only some new and striking aspects in their biology and distribution are outlined here.

Parasitism in different plants.—The great bulk (over 95 per cent.) of parasites has been recovered from one species of wild plant, namely, *T. rhomboidea*, the most important food plant of the weevil. Levels of parasitism of course display seasonal trends but sustained high levels both in number and variety of parasites have been observed in this species. *Sida acuta* stands probably second in this respect. All others show only occasional and intermittent parasitism as seen from mean rate of parasitism (*vide* data on rate of parasitism in respect of each species of wild plant).

Rate of parasitism in alternate food plants

Plant species	Parasitism	
	Maximum %	Mean %
T. rhomboidea	44·64	12·24
S. acuta	66·60	2·75
S. spinosa	27·60	2·10
C. olitorius	16·00	0·68
Malvastrum coromandelianum	15·30	0·72
P. zeylanica	18·10	1·40
H. vitifolius	40·00	1·30
Althæa rosea	2·28	0·76

Entedon pempheridis Ferr. (Eulophidæ) (Fig. 3, Plate XXV)

This species is the most numerous among alternate food plant parasites. It is a metallic blue and green chalcid of an average length of 2·5 mm. It is a primary, larval parasite and the only endophagous species in the lot.

Distribution and host records.—It is widely distributed in Coimbatore, Shemmedu, Thondamuthur, Wallayar and Malabar. It attacks *Pempheres* in a variety of host plants such as *T. rhomboidea*, *S. acuta*, *C. olitorius*, *H. esculentus*, *H. vitifolius* and *Malvastrum*. Other hosts of the species are *Apion* sp. in *Corchorus olitorius*, *C. trilocularis* and *Lobotrachelus* sp. in *H. manihot*. It is totally absent in cotton fields.

Life cycle.—Egg and Larval period 8–11 days; Prepupal period 1–3 days; Pupal period 8–12 days; Maximum adult longevity 46 days, averaging 25·5 days.

Sesaonal history.—These are found in abundance from August–March, comparatively rare in other months.

Dinarmus coimbatorensis Ferr. (Pteromalidæ) (Fig. 1, Plate XXV)

This is a dark green insect of about 4 mm. in length. The species is a primary, ectophagous, larval parasite. It stands second in importance in view of numbers and efficiency.

Distribution and host records.—It is probably widely distributed throughout the southern districts having been taken from Coimbatore, Malabar, S. Canara and Tanjore. It attacks *Pempheres* in *T. rhomboidea*, *S. acuta*, *S. spinosa*, *C. olitorius*, *H. esculentus* and probably *Urena lobata*. The only other host of the species is *Hypolixus*. It is absent in cotton fields. Recently a few specimens have been noted in the same.

Life cycle.—Total period 17–21 days; Preoviposition period 2 days; Egg period 1–2 days; Larval period 5–8 days; Prepupal period 1–3 days; Pupal period 10–12 days; Maximum adult longevity with food 42 days.

Seasonal history.—These occur in some numbers from July–December. In February–March they again appear in small numbers. At other periods they are rare.

Bruchocida orientalis Crawford

This species was first collected as a pupa from a *Pempheres* grub in *T. rhomboidea* in July 1937. It has since been recovered on another occasion. It is a primary ectophagous larval parasite. Life-history is not known. It has been recorded from Bangalore as a parasite of *Bruchus chinensis*.

Eurytoma sp. and *Aximopsis* sp.

These two parasites were obtained from *Pempheres* grubs infesting *Hibiscus esculentus* in Malabar. Only one or two individuals of each species have been bred out. Because of their rarity and unimportant status no data on their biology have been obtained.

Spathius labdacus Nixon (Braconidæ) (Fig. 4, Plate XXV)

This is the third important parasite of the weevil in alternate host plants. This reddish-brown winged Braconid is the largest of all weevil parasites. It is a primary, ectophagous, larval parasite.

Distribution and host records.—The species has been collected from Coimbatore and surroundings, Malabar and S. Arcot. The only known host is *Pempheres* infesting *P. rhomboidea*. Recently one specimen, probably belonging to this species, has been obtained from *Hibiscus esculentus*.

Life cycle.—Total period 17–22 days; Preoviposition period 3 days; Egg period 1–2 days, averaging 1–4 days; Larval period 4–6 days, averaging 4·4 days; Prepupal and Pupal periods 13–16 days, averaging 14 days; Maximum adult longevity with food 121 days.

Seasonal history.—It occurs in the season September–January in Coimbatore and February–March in Malabar.

Rhaconotus cleanthes Nixon (Braconidæ) (Fig. 2, Plate XXV)

This slender deep brownish braconid is the fourth important parasite of the pest in alternate food plants. It is also a primary parasite attacking the grubs externally.

Distribution and host records.—It has been taken from Coimbatore and Malabar. *Pempheres* is perhaps its only host. It attacks the same in different plants, namely, *T. rhomboidea* and *S. acuta*. The species is absent in cotton fields.

General biology.—Oviposition is infrequent and erratic in cages. The mature larva can be distinguished from other braconids from its slender elongate (almost worm-like) cylindrical form and by the brownish tinge.

Seasonal history.—These occur mostly in October–November.

Rhaconotus menippus Nixon

The general form, size and life-history are in most respects similar to those of the former species.

Distribution and host records.—Its distribution appears to be so far limited to Coimbatore. Occasionally it attacks *Pempheres* mainly in *C. olitorius* and *T. rhomboidea*. It is absent in cotton fields. A single case of attack in cotton has been recently obtained probably as a result of importing wild host plants. *Hypolixus truncatulus* is its other host.

Geomermis indica Steiner (Nematoda) (Fig. 5, Plate XXV)

Besides insect parasites, a nematode parasite has been occasionally observed parasitising *Pempheres* grubs and later stages infesting *T. rhomboidea* and *Malvastrum* during the season September–January.

Conclusion

The percentage of parasitism in cotton fields is apparently too low to exercise any adequate control of *Pempheres*. Their numbers are extremely insufficient for the purpose. The host-parasitic ratio is capable of being improved by artificial multiplication and timely liberation of at least one species—*Spathius critolaus*. Beneficial results may follow this procedure. The more promising line of action, however, is to introduce into cotton fields the parasites of its pre-economic habitat by direct importation of parasitised material as also by laboratory breeding and manipulation, should the species prove amenable in this regard. Success in this or any similar project can never be assured. Attempts in these directions are desirable.

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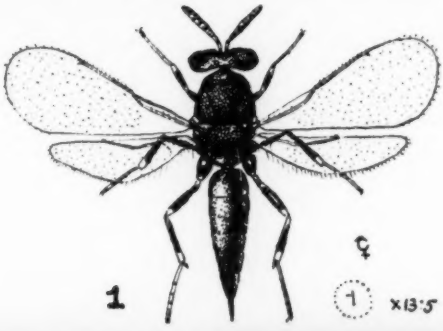

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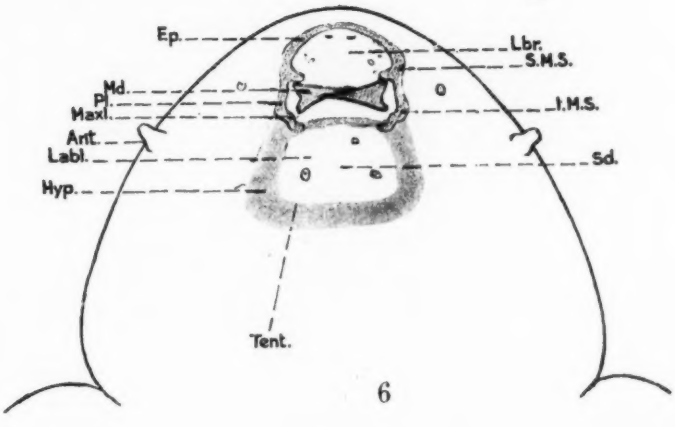

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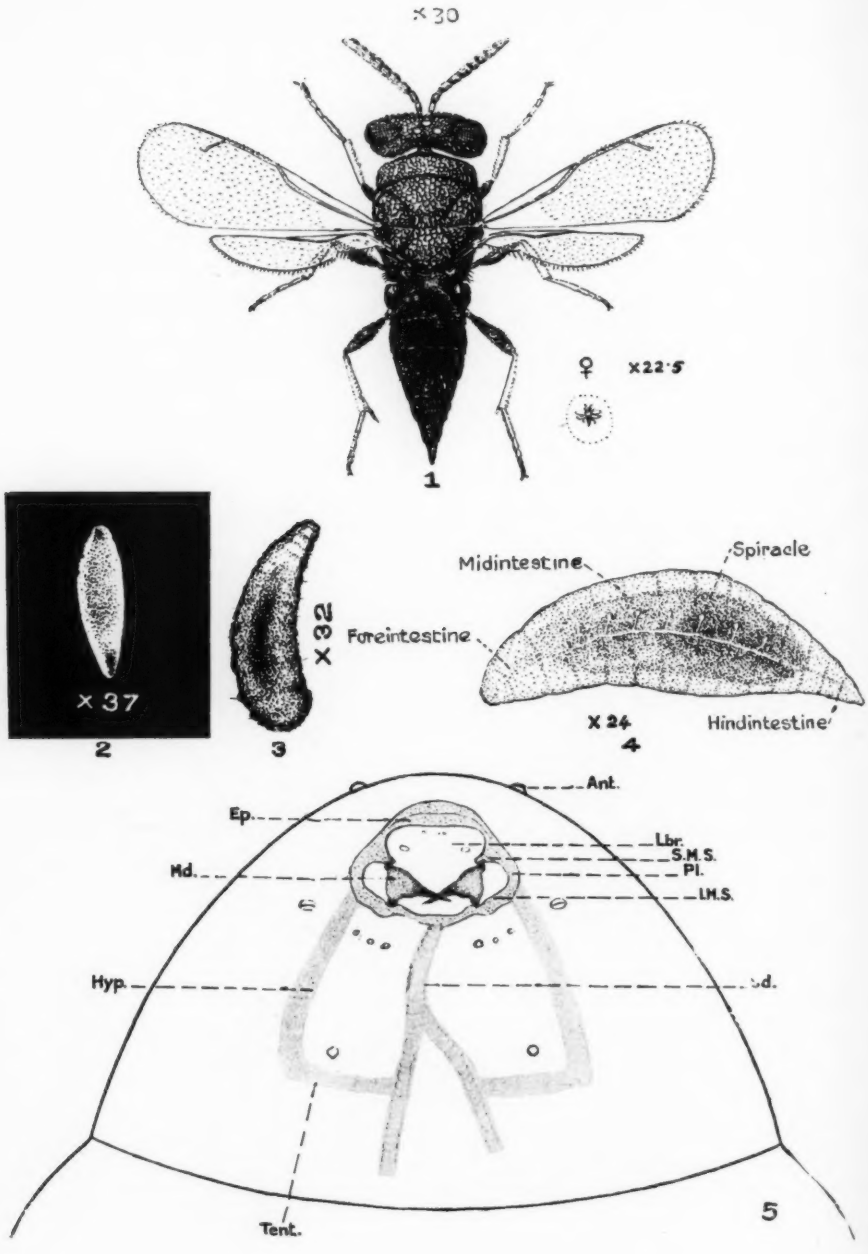
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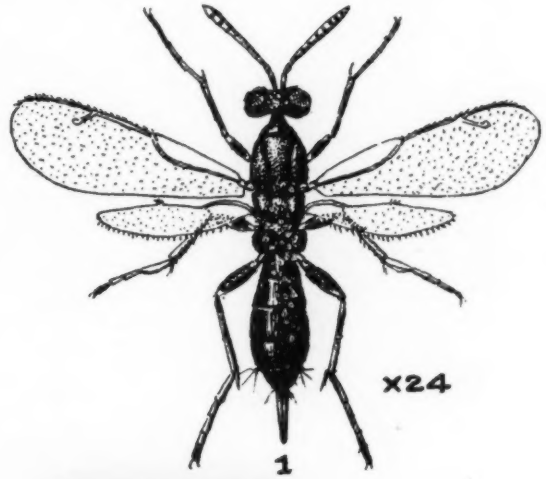
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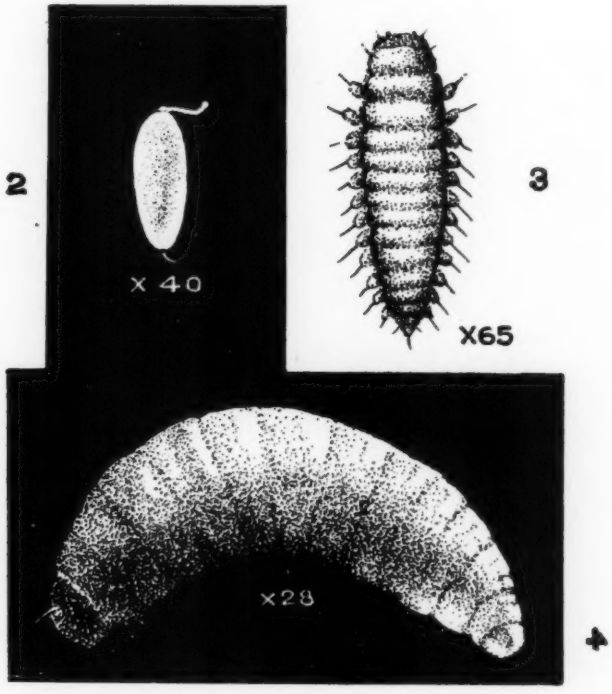
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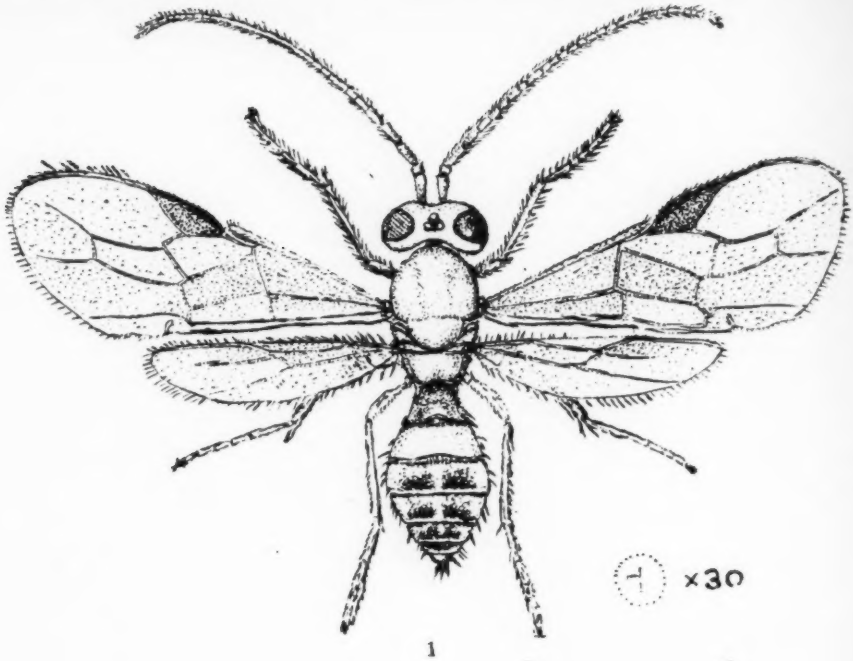

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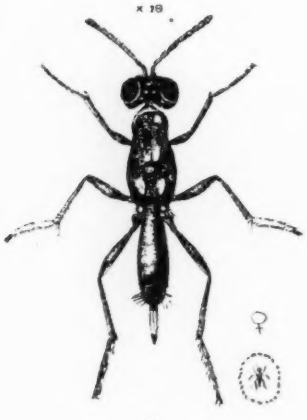

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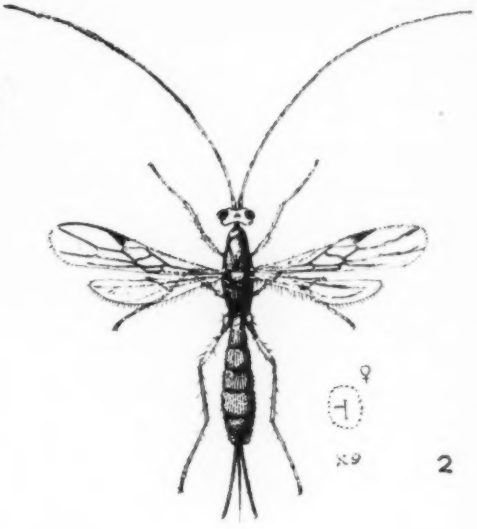

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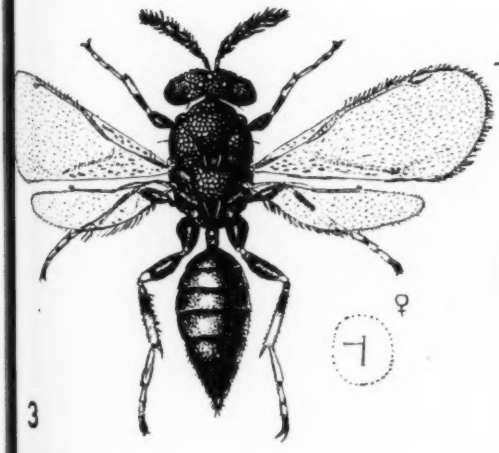

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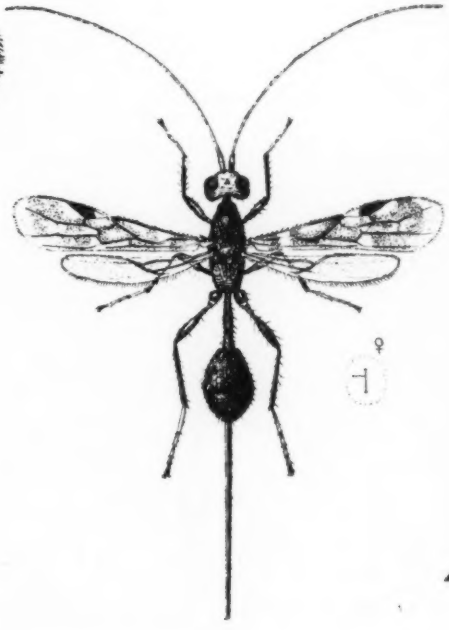

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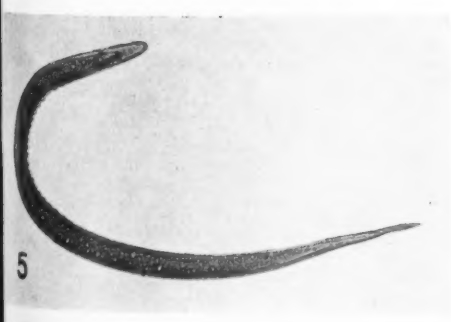

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EXPLANATION OF PLATES

PLATE XXI. *Euderus pempheriphila*

FIG. 1. Adult.

FIG. 2. Egg.

FIG. 3. Mature larva (side view).

FIG. 4. Prepupa (dorsal view).

FIG. 5. Pupa (dorsal view).

FIG. 6. Head and mouth parts of larva (nearly full-grown) (ventral view).

Ep., epistoma; *Hyp.*, hypostoma; *I.M.S.*, inferior mandibular strut; *Lab.*, labium; *Lbr.*, labrum; *Max.L.*, Maxillary lobe; *Pl.*, pleurostoma; *Sal.d.*, salivary duct; *S.M.S.*, superior mandibular strut; *Tent.*, tentorium; *Md.*, mandible; *Ant.*, antenna; *Lab.L.*, labial lobe.

PLATE XXII. *Aplastomorpha calandræ*

FIG. 1. Adult.

FIG. 2. Egg.

FIG. 3. Newly hatched larva (dorsal view).

FIG. 4. Mature larva (side view).

FIG. 5. Head and mouth parts of full-grown larva (ventral view).

Ep., epistoma; *Hyp.*, hypostoma; *I.M.S.*, inferior mandibular strut; *Lab.*, labium; *Lbr.*, labrum; *Max.L.*, maxillary lobe; *Pl.*, pleurostoma; *Sal.d.*, salivary duct; *S.M.S.*, superior mandibular strut; *Tent.*, tentorium; *Md.*, mandible; *Ant.*, antenna; *Lab.L.*, labial lobe.

PLATE XXIII. *Eupelmus urozonus*

FIG. 1. Adult.

FIG. 2. Egg.

FIG. 3. Newly hatched larva (dorsal view).

FIG. 4. Mature larva (side view).

PLATE XXIV

FIG. 1. *Microbracon* n.sp. (Adult).

FIG. 2. *Spathius critolaus* (Adult).

FIG. 3. *Eupelmella pedatoria* (Adult).

PLATE XXV

FIG. 1. *Dinarmus coimbatorensis* (Adult).

FIG. 2. *Rhaconotus cleanthes* (Adult).

FIG. 3. *Entedon pempheridis*.

FIG. 4. *Spathius labdacus*.

FIG. 5. *Geomermis indica* (Nematode).

THE DEVELOPMENT OF THE MALE GAMETE IN *CYMBIDIUM BICOLOR* LINDL.

By B. G. L. Swamy

Received September 23, 1941
(Communicated by Prof. A. Subba Rau)

Introduction

Cymbidium bicolor belongs to the tribe *Vandeæ* and sub-tribe *Cymbidieæ* of Orchidaceæ of Bentham and Hooker; it is found growing wild in the Western Ghats and is planted in gardens for its flowers. It flowers in the month of May. An account of the development of the male gamete is presented here.

Materials and Methods

Material for the present investigation was collected in gardens round about Bangalore, and was fixed in Bouin's fluid, and formalin-acetic alcohol. The removal of perianth from young buds facilitated infiltration. Sections of 8 to 12 μ in thickness were cut and stained in Heidenhain's iron-alum hæmatoxylin, with Eosin in clove oil as counter-stain.

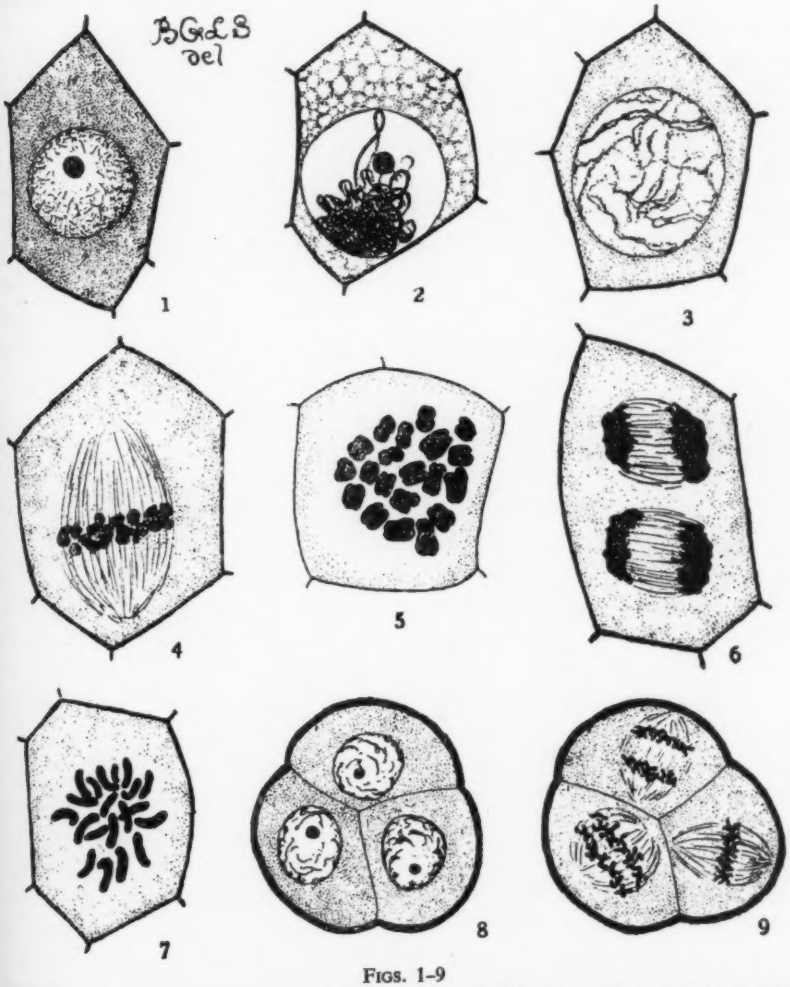
For studying the cytology of the pollen tube the following technique was adopted. The flowers were artificially pollinated at various intervals. The enlargement of the gynostegium indicated successful pollination. The entire column was removed, dissected under a microscope stained with picronigrosin, and was mounted in lactophenol.

Permanent preparations of the pollen tubes were done according to the method adopted by Kausik (1939) for making the whole mounts of endosperm from the seeds of *Grevillea robusta*. Eosin in clove oil was used in some cases for counter-staining.

Microsporogenesis

The microspore mother cell at the resting stage (Fig. 1) is polygonal in outline with rich protoplasmic contents and a prominent nucleus. The nucleus enlarges in size and various phases of reductional division could be distinctly followed. Synapsis and other subsequent changes take place in the usual manner and the threads pass into the Synizesis (Fig. 2). The pachytene threads (Fig. 3) show double nature distinctly. With the disappearance of the nuclear membrane at late diakinesis, the chromosomal pairs arrange themselves along the equatorial plate (Fig. 4). 20 Chromosomes

were counted in the metaphase plate (Fig. 5). Second division (Fig. 6) is simultaneous without a period of interkinesis. The disposition of the II division spindles is either parallel or at right angles to each other. The four microspores are arranged as quartets (Fig. 8), which are either isobilateral or tetrahedral.

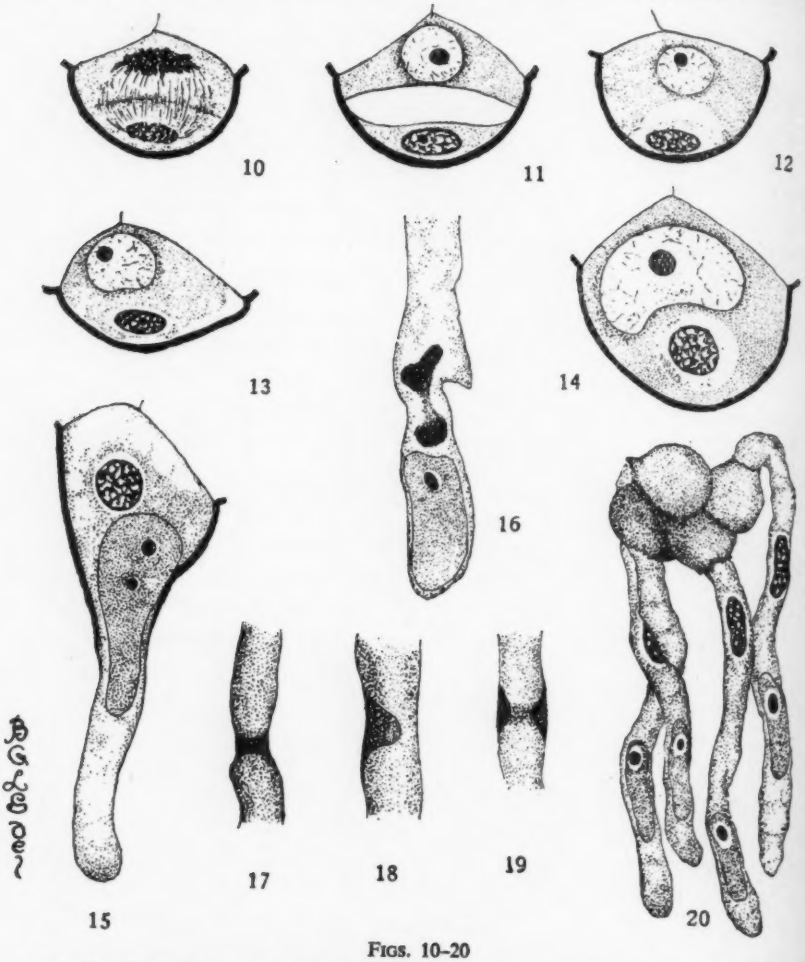


FIGS. 1–9

FIG. 1. Microspore mother cell resting. FIG. 2. Synizesis. FIG. 3. Pachytene stage. FIG. 4. Metaphase I. FIG. 5. Polar view of metaphase I. FIG. 6. Telophase II. FIG. 7. Polar view of metaphase II. FIG. 8. Tetrahedral quartet. FIG. 9. Division of the uninucleate microspore. All figures magnified 1,800 times except Figs. 5 and 7 which are magnified 1,200 times.

Even in the polar views of the metaphase plates of the II division (Fig. 7) 20 chromosomes were distinctly counted thus confirming the haploid chromosome number already mentioned.

The microspore slightly enlarges in size and the nucleus undergoes division (Fig. 9). The division is followed by wall formation (Fig. 10), which can



FIGS. 10–20

FIG. 10. Early telophase during the division of the uninucleate microspore. Note the deposition of the cell wall. FIG. 11. The two daughter cells derived from the stage shown in Fig. 10 moved apart. FIG. 12. Disappearance of the cell membranes and an early stage in the inpushing of the generative nucleus. FIG. 13. A later stage than the one shown in Fig. 12. FIG. 14. Microspore with two nuclei—the inner tube nucleus and the outer generative nucleus. Note the

empty space around the generative nucleus. FIG. 15. Germination of the microspore. FIG. 16. Division of the generative nucleus in the tube. FIGS. 17–19. Stages in the formation of plugs. FIG. 20. All the individuals of a quartet germinating without separation. All figures magnified 1,800 times except Fig. 20 which is magnified 800 times.

be made out during the late telophase. But later the wall disappears and a clear space separates the two cells (Fig. 11).

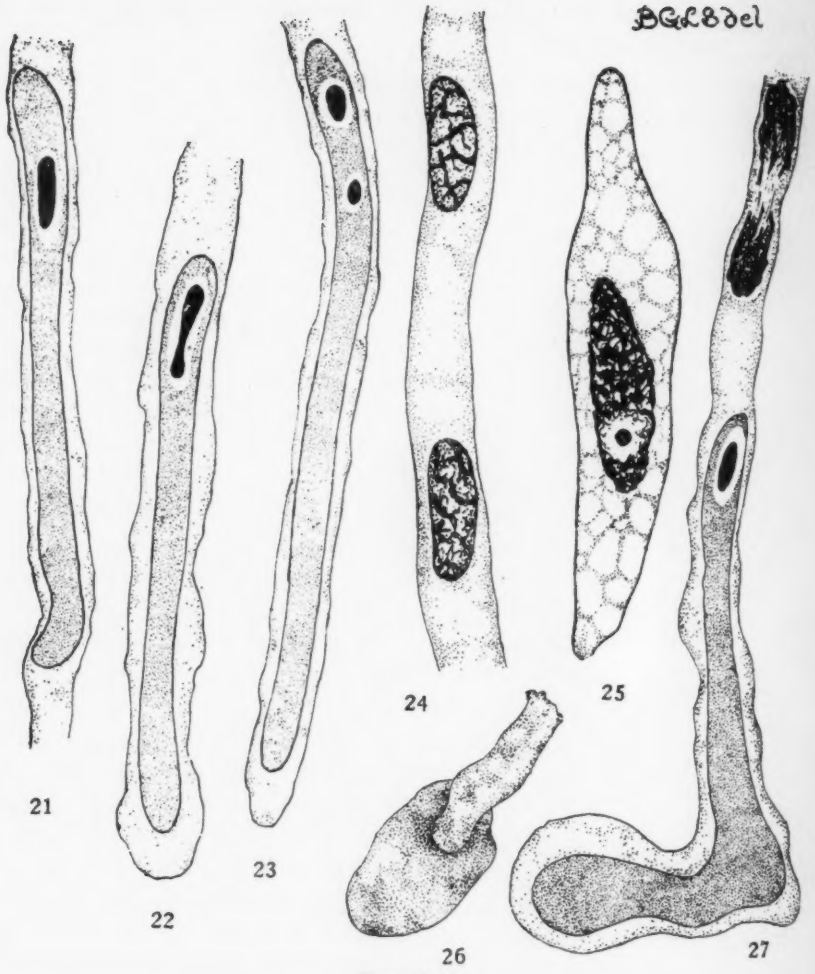
The generative cell which is towards the outside of the pollen grain is closely adpressed to the wall of the microspore. It is lenticular in shape and shows thick chromatin contents. Before germination of the microspore the following stages of development have been observed. The lenticular generative cell becomes slightly spherical and gradually becomes spindle-shaped (Fig. 12) and the plasma of the tube cell grows around the generative cell (Fig. 13), which becomes engulfed in the tube cell (Fig. 14). In this process the nucleus of the tube cell becomes semilunar, possibly due to the pressure of the generative cell on the contiguous side. A clear space can be observed around the inpushed generative nucleus. Similar phenomena is reported by Wylie (1904) in *Elodea* and in *Vallisneria* by Witmer (1937).

The mature pollen grain in *Cymbidium bicolor* has two nuclei—a very darkly staining globose generative nucleus, usually lying toward the outer edge of the quartet and a faintly staining, much larger tube nucleus with one or, occasionally two, round nucleoli. The individuals of the quartet never separate from one another, but remain together even after the quartets have germinated (Fig. 20).

Pollinia begin germinating only after pollination. The gynostegium enlarges nearly three or four times its original size. The germ pore which is indistinct becomes evident at the time of germination. During germination it was noticed that many of the quartets in the centre of the pollinial mass degenerated. Probably these contribute food to the germinating ones.

The pollen tube grows in length and the tube and the generative nuclei enter into it (Fig. 15). In most of the cases observed the tube nucleus enters first followed by the generative nucleus. The pollen tube traverses through the gynostegium and reaches the ovarian cavity 3 to 4 days after pollination. The cells of the conducting tissue contain dense cytoplasm which is vacuolate (Fig. 25). The cytological details of the pollen tube can be observed by dissecting the gynostegium as already suggested under material and methods.

The tube nucleus in the microspore before germination measures 8 to 10 μ and after entering the pollen tube becomes cylindrical in shape (Figs. 21–23) and very much elongated reaching in some cases 8–10 times its original length. In one case it was found to measure 98 μ. The tube nucleus does not



FIGS. 21–27

FIGS. 21–23. Stages illustrating the fragmentation of the nucleolus of the tube nucleus. FIG. 24. Two bean-shaped male nuclei lying in the pollen tube. FIG. 25. A cell of the conductive tissue of the stylar region. FIG. 26. Tip of a pollen tube developed into a cyst. FIG. 27. An earlier stage of the division of the generative nucleus than shown in Fig. 16; note the tendency, of the tip of the pollen tube to swell to form a cyst. All figures magnified 1,800 times except Fig. 25 which is magnified 800 times.

stain heavily with hæmatoxylin but shows only diffuse granular contents. Another interesting feature is that the nucleus appeared to be flexible since it showed corresponding contour with the pollen tube (Fig. 27).

Fragmentation of the nucleolus was also observed and the stages are represented in Figs. 21–23.

The generative nucleus in the pollen tube enlarges 4–5 times its original length and undergoes division (Figs. 16 and 27). Prior to dividing the generative nucleus shows thick chromatin contents and the clear space around it disappears. Details of this division could not be followed. A very careful examination of suitable stages were made in order to know if a separating membrane or cell wall was laid down between the separated male nuclei, but no indication of this could be made out (Figs. 16 and 27). Absence of such a membrane is reported by Herrig (1919) in *Echeveria Desmetiana* and by Piech (1924) in *Scirpus lacustris*. Witmer (1937) has seen in *Vallisneria spiralis* that in " some cases no cell plate is any longer discernible ". The separated male nuclei move still farther apart from each other and organize into well-defined typically bean-shaped nuclei with rich chromatin (Fig. 24).

The wall of the pollen tube is uniform and smooth except when it sometimes enters into the tissue of the gynostegium when it assumes rugged contours and narrow twists. The tip of the pollen tube is usually round and blunt.

Sometimes the tip of the tube shows a tendency to swell and form a cyst (Figs. 26 and 27). In such cases the tube nucleus was found to enter the cyst. Wylie (1904) in *Elodea Canadensis* observed similar teminal cysts in some pollen tubes.

In the older regions of the pollen tube, formation of " plugs " is of common occurrence. Such plugs are reported in *Gossypium* by Iyengar (1938). The development of plugs was followed in detail. They arise on the inner surface of the pollen tubes as small thickened regions and grow towards the centre, finally obliterating the pollen tube cavity at that region (Figs. 19 and 17). Occasionally the pollen tube cavity was seen to be obliterated by the plug arising from only one side of the tube also as shown in Fig. 18. According to Iyengar (1938) the function of these plugs is to prevent the backward flow of nutriment.

Summary

(1) The haploid chromosome number was determined from the metaphase plate of the heterotypic division to be 20.

(2) The uninucleate microspore gives rise to two definite cells following nuclear division, the generative cell and the tube cell. A clear space appears between the two cells, their adjacent cell membranes disappear and the generative nucleus shifts itself back and is engulfed into the tube cell.

(3) The individuals of the quartet do not separate, but remain intact.

(4) Pollinia germinate only after pollination. The tube nucleus elongates in the pollen tube upto 80 to 90 microns. The nucleolus of the tube nucleus occasionally undergoes fragmentation.

(5) The generative nucleus divides in the tube to form two male nuclei.

(6) Cyst formation at the tip of the pollen tubes is of common occurrence and development of plugs within the pollen tubes has been observed in many cases.

I am deeply indebted to Dr. M. A. Sampathkumaran, M.A., Ph.D., S.M., and Mr. S. B. Kausik, M.SC., for their guidance and discussion.

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MEGASPOROGENESIS AND ENDOSPERM FORMATION IN *ERIODENDRON ANFRACTUOSUM* D.C.

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(*Department of Botany, Central College, Bangalore*)

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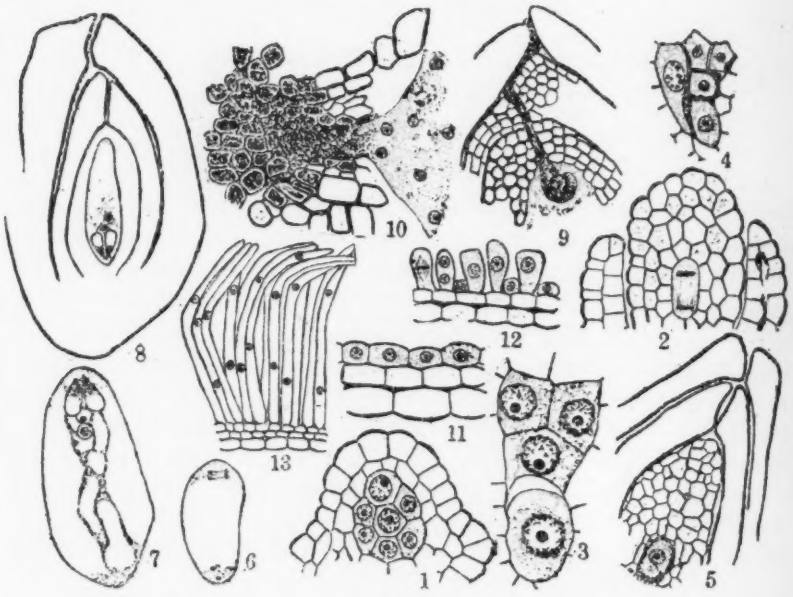
The white silk cotton tree or Kapok tree of the Dutch, is cultivated in India for the silky floss obtained from the fruits. The genus *Eriodendron* is included under the family *Malvaceæ* by Bentham and Hooker (1895), whereas both Engler and Prantl (1928), and Hutchinson (1926) treat it separately under the *Bombacaceæ*. The presence of egg-shaped stem, smooth pollen and other characters are taken as the basis for distinguishing *Bombacaceæ* from *Malvaceæ*. Literature on embryological studies reviewed by Schnarf (1931) and Schürhoff (1926), shows that no work has so far been forthcoming on the family *Bombacaceæ*. Schnarf (1931) also suggests that embryological studies on the *Elæocarpaceæ* and *Bombacaceæ* might throw some light on the group Columniferæ. The present work was undertaken with a view to investigate the development of the female gametophyte and endosperm formation in *Eriodendron anfractuosum* D.C.

The flowers were collected during the months of January and February, and fixed in Flemming's weak solution and Allen's modified Bouin. Sections of 8 to 14 μ thickness were cut, and stained with Heidenhain's iron-alum hæmatoxylin with orange G as counter-stain in some cases.

The ovules are anatropous and bitegumentary. The integuments take their origin as rings of cells at the base of the growing mass of nucellar tissue. In later stages the integuments become massive and are flattened at the apex. The micropyle is narrow and zig-zag (Fig. 9) as in *Lagerstræmia* reported by Joshi and Venkateswarlu (1936).

The archesporium is hypodermal in origin and can be differentiated by its conspicuous nucleus and cytoplasm. It cuts off a parietal and megaspore mother cell. The parietal cell by further divisions gives rise to a massive tissue. Consequently the megaspore mother cell becomes deep-seated. At one nucleate embryo-sac stage 9 to 12 layers of parietal cells can be observed

(Fig. 5). The development of more than one archesporium has been observed in many cases (Fig. 1). In one case two archesporial cells showed development, one of them up to the tetrad stage, and the other up to diakinesis in the megaspore mother cell (Fig. 4). The occurrence of more than one mature embryo-sac has so far not been observed.



FIGS. 1–13

FIG. 1. Showing the development of seven archesporial cells. × 900. FIG. 2. Megaspore mother cell in anaphase. × 400. FIG. 3. T-shaped tetrad showing the enlarging chalazal megaspore. × 1800. FIG. 4. Development of two megaspore mother cells, one showing diakinesis and the other a T-shaped tetrad. × 900. FIG. 5. One-nucleute embryo-sac. × 400. FIG. 6. Division of the two-nucleate embryo-sac to form the four-nucleate embryo-sac. × 400. FIG. 7. Mature embryo-sac showing the egg apparatus. × 800. FIG. 8. Reversed polarity in an eight-nucleate embryo-sac. × 800. FIG. 9. Fertilization. × 800. FIG. 10. Chalazal end of the embryo-sac after fertilization. × 900. FIGS. 11, 12 and 13. Various stages in the development of the cotton from the carpellary wall. × 800. FIG. 13. × 160.

The megaspore mother cell enlarges and undergoes the usual heterotypic (Fig. 2) and homotypic divisions and forms four megaspores. In all cases observed a T-shaped tetrad is formed. The chalazal megaspore enlarges and forms the embryo-sac. The large number of parietal cells above the megaspore degenerate and contribute nutrition. The development of the embryo-sac conforms to the normal type (Figs. 6, 7). The egg apparatus is organised at the micropylar end. The synergids are pear-shaped

with a basal vacuole. The antipodals show early degeneration and the polars fuse even before the organisation of the egg apparatus.

A single case of embryo-sac reversal has been observed (Fig. 8). The egg apparatus is organised at the chalazal end. Similar instances of embryo-sac reversals have been reported in *Woodfordia floribunda* and *Lagerstræmia* (Joshi and Venkateswarlu, 1935), in *Rhopalocnemis phalloides* (Lotsy, 1901), in *Fuchsia Marinka* (Tackholm, 1915), *Lindelofia longiflora* (Svensson, 1925), *Atamosco texana* (Pace, 1913), *Saccharum officinarum* (Dutt and Subba Rao) and others.

The pollen tube enters through the zig-zag micropyle and crushes the synergids in its passage (Fig. 9). The surface of the pollen tube is minutely spinescent. The actual fusion of the egg and the male nucleus was not observed.

The endosperm nucleus undergoes large number of divisions and the nuclei become distributed along the periphery. Wall formation takes place only in the later stages. During post-fertilization stages the chalazal end of the embryo-sac becomes haustorial in function (Fig. 10). The surrounding cells are depleted of their contents and thus supply nutrition to the developing embryo. The chalazal end of the embryo-sac penetrates into the nucellar tissue. A similar case of the piercing of the chalazal end of the embryo-sac in the post fertilization stages has been recorded in *Suriana maratima* (Anantaswamy Rau, 1940).

The development of the cotton has been studied in detail (Figs. 11, 12, 13). After the formation of the endosperm the cells of the carpel bordering the locule become active. The nucleus enlarges in size, and the cells contain dense cytoplasm. As development proceeds the cells elongate and become tubular. The nucleus undergoes one division with the result the cells of the floss are binucleate. In mature fruits the floss fills up the space between the seeds and the carpellary wall.

Conclusions

The study of the female gametophyte and endosperm formation in *Eriodendron anfractuosum* reveals a normal type of development with some variations. The occurrence of multiple archesporium, T-shaped tetrads and a large number of parietal cells in early stages are noteworthy features. A single case of embryo-sac reversal has been recorded in *Eriodendron anfractuosum*. Similar instances of embryo-sac reversals have been reported by various investigators. Whether fertilization takes place in such cases is not known.

The development of cotton, or the floss as it is called, is very interesting. It has been shown to develop from the epidermal layer of the inner carpellary wall. Studies on the development of cotton in *Gossypium* where it is formed from the epidermal layer of the testa (Turner, 1929), have shown that the cells of the cotton are uninucleate. Barritt (1932) has shown that the cotton in *Gossypium* is composed of only uninucleate cells. Mitoses, often observed in the epidermal layer, are shown to be incipient ones. In the floss of *Eriodendron* the cells are invariably binucleate, the nucleus dividing at very early stages of differentiation of cotton fibres. Gamble (1902) remarks that the floss in *Eriodendron* serves only as a sort of packing material for the seeds, and this seems probable by the type of its development from the carpellary walls. But observations showed that after the bursting of the capsule the seeds become surrounded by small portions of cotton, and this helps a good deal in the dispersal of the seeds.

Summary

1. The development of the female gametophyte and endosperm formation in *Eriodendron anfractuosum* has been studied.

2. Ovules are bitegumentary, anatropous with a zig-zag micropyle. The archesporium is hypodermal, and cuts off a parietal cell. Development of more than one archesporium has been observed.

3. The four megaspores formed are arranged as T-shaped tetrads.

4. The development of the embryo-sac conforms to the normal type. Antipodals show early degeneration.

5. A case of embryo-sac reversal has been noticed.

6. Endosperm is nuclear, and during post-fertilization stages the chalazal end becomes haustorial in function.

7. The floss is formed from the inner epidermal layer of the carpel. The cells of the floss are binucleate.

In conclusion, the authors wish to acknowledge their indebtedness to Dr. M. A. Sampathkumaran, M.A., Ph.D., for guidance and encouragement during the course of this work.

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A NEW SPECIES OF PUCCINIA ON *OCIMUM ADSCENDENS*

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(Communicated by Prof. A. Subba Rau)

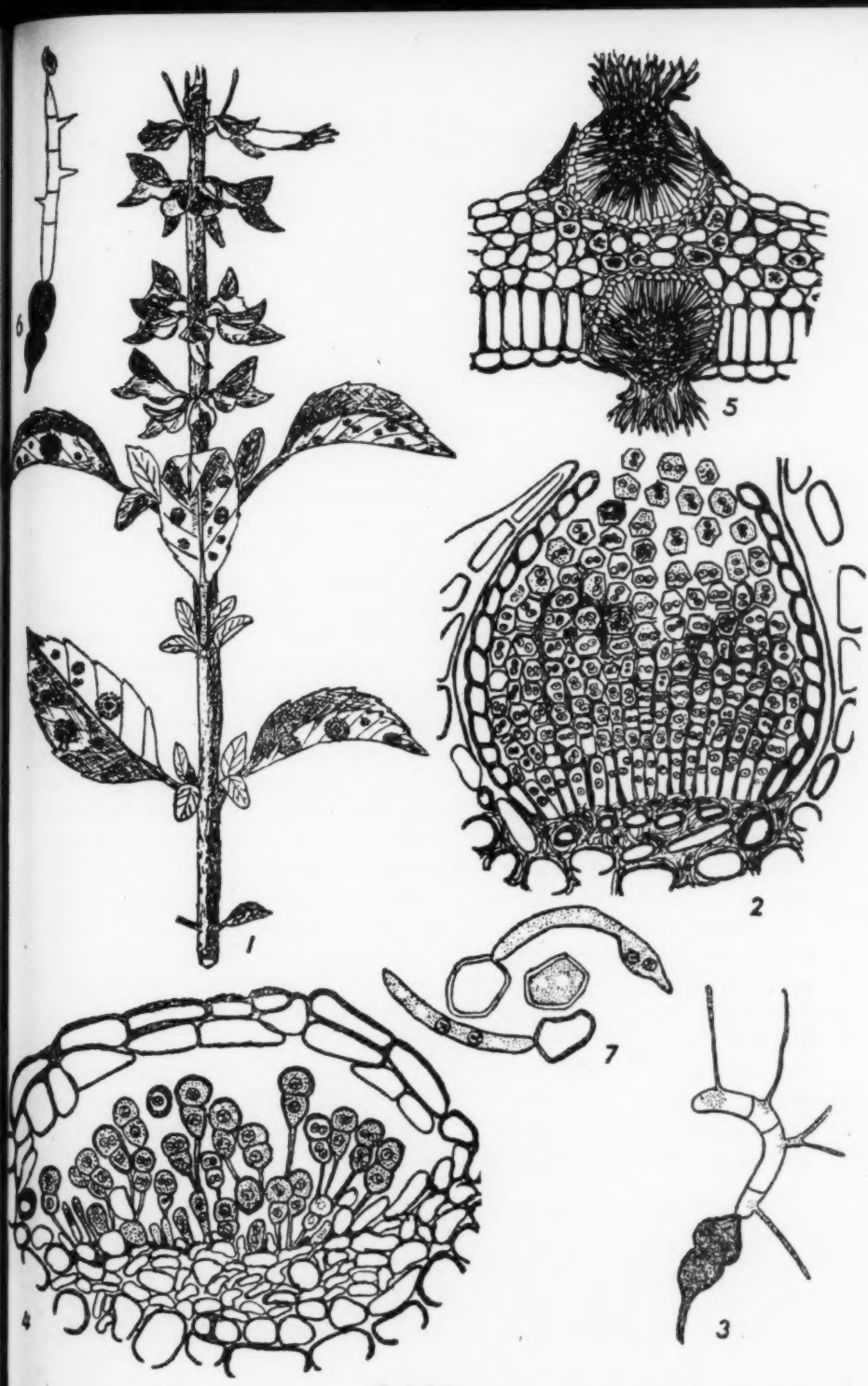
Introduction

The occurrence of rust fungi on plants belonging to the tribe *Ocimoidæ* of Labiatæ of Bentham and Hooker (1885) is not widespread. *Aecidium Plectranthi* Barc. and *Coleosporium Plectranthi* Barc. have been recorded on *Plectranthus cæsta* and *P. gerardianus* (Barclay, 1890). *Eriosporangium Hyptidis* (Curtis) Arth. is found to occur on leaves of *Hyptis* sp.; An *Aecidium* on *Ocimum canum* was collected by McRae near Koilpatti, Madras Presidency, which was identified at first by Sydow (1914) as *Aecidium Ocimi* P. Henn. Later he made the same collection the type of a new species *Aecidium leiocarpum* Syd. (Sydow, 1917) due to the fact that the Koilpatti specimen had smaller peridial cells, smaller and almost smooth spores. *Aecidium Ocimi* first recorded by Hennings has not so far been known to occur in India and it has been mentioned to occur in India by Butler and Bisby (1931) by mistake. An account of the *Puccinia* sp. collected by the author on *Ocimum adscendens* near Bangalore is presented here.

Ocimum adscendens is a perennial weed growing in arid places along with herbaceous undergrowth. The fungus is found parasitising the leaves and stems in some cases, forming concentric yellow patches (Fig. 1). The infected portions become slightly swollen up due to hypertrophy. Three spore forms are found to occur in the life-cycle of this rust fungus; pycnia, aecia and telia.

Pycnia are flask-shaped and orange yellow in colour. They are subhypodermal and are developed on both the surfaces of the leaf. A well-developed ostiole with paraphyses, nectar and spores can be clearly observed (Fig. 5).

Aecia are also amphigenous, cupulate and covered with a well-developed peridia (Fig. 2), characteristic of the genus *Puccinia*. During dry weather conditions, the spore column is not broken, but become enclosed in the elongating peridial sheath and resemble the rostelia type of aecia present in *Gymnosporangium*. Aeciospores are in chains separated by the sterile intercalary



FIGS. 1–7

FIG. 1. Twig of *Ocimum adscendens* showing infection in concentric patches. × Natural size. FIG. 2. Camera lucida drawing of an æcium showing chains of spores and well-developed

peridia. × 400. FIG. 3. Germination of the teliospore showing whip-like sterigma without any sporidial development. × 400. FIG. 4. Camera lucida drawing of a teliosorus with spores in various stages of development. × 400. FIG. 5. Showing the development of pycnia on both the surfaces of the leaves. × 160. FIG. 6. Germination of a teliospore. × 400. FIG. 7. Showing the germination of æciospores. × 800.

cells. The spores are polyhydral, smooth walled and binucleate. The germ pore is indistinct and becomes visible only during germination. The spores measure $17 \times 14\,\mu$, and the peridial cells $22 \times 17\,\mu$.

Aeciospores were germinated and stained by the method previously suggested by the author (1940). The nuclei migrate into the germ tube which later on becomes septate (Fig. 7). The tip of the germ tube in most cases forms an appresorium.

Telia are formed in the month of October following the development of aecia. Initials of telia are subhypodermal, formed by the concentration of mycelia. Long slender binucleate hyphæ are differentiated, the tips of which become bulbous and cut off a cell. The tip cell forms a two-celled teliospore by the formation of a transverse septa. Mature spores are uninucleate, thick-walled, and deep brown in colour. The spores are stipitate and persistent, with a germ pore in each cell (Fig. 4). They measure $25 \times 11\,\mu$.

Mature teliospores germinate without a period of rest being necessary (Fig. 6). Both the spores of a teliospore germinate simultaneously, and form sporidia within 24 hours. Teliospore material collected late in the season had only empty spores and could not be germinated with success even when they showed contents. The cells of the promycelia are uninucleate as also the basidiospores. Secondary sporidia have not so far been observed. Basidiospores are thin-walled and spherical. They readily germinate under moist conditions, and measure $10 \times 8\,\mu$. Abnormal types of germinations, mostly attributed to excess of moisture, were observed even under normal conditions. The sterigmata developed into long filamentous structures without any basidiospore formation (Fig. 2). Branchings of sterigmata were found to be of frequent occurrence. In one case both the branches had developed sporidia at the tip. It is not certain whether more than four spores could develop in such cases. Submerged spores elongated indefinitely, without septation, and remained uninucleate.

Sporidial Infection

Teliospores were germinated in moist chambers and placed on young *Ocimum adscendens* leaves, which were previously sprayed with water. The inoculum was transferred on to the leaves by means of a camel hair brush. The infected plants were placed in moist chambers for 24 hours. The first

sign of infection became visible after 8 days as a tiny yellow speck. The infected spot gradually spread out to an yellow patch, and pycnia were developed after 16 days. Aecial pustules were observed after 22 days replacing the pycnia which are evanescent.

Conclusions

The rust on *Ocimum adscendens* is an autœcious form, as indicated by the infection experiments, with three spore forms pycnia, æcia and telia. *Aecidium leiocarpum* has been found to occur in India on *Ocimum canum*, whereas it is not known whether *Aecidium Ocimi*, first recorded by Hennings, is present in India or not. Comparative studies on æciospore measurements indicated that the æciospore of the rust fungus on *Ocimum adscendens* and *O. canum* were similar in all respects. The æciospores in both the cases measured $17 \times 14\,\mu$ and were polyhydral and smooth-walled. On the other hand the æciospores of *Aecidium Ocimi* are known to measure $24 \times 20\,\mu$, and are evidently bigger in size than those of *A. leiocarpum*. An *Aecidium* on *Ocimum adscendens* obtained from the Mycological Laboratory,* Agricultural Research Station, Coimbatore, for comparison was found to be the æcial stage of the *Puccinia* species on *Ocimum adscendens*. From these considerations it is quite evident that the teliospores recorded on *Ocimum adscendens*, is the perfect stage of *Aecidium leiocarpum* Syd., first collected by McRae near Koilpatti, Madras. The author proposes to make a new combination as *Puccinia leiocarpum* (Syd.) Thirumalachar, with three spore forms 0, 1 and III.

Description of the Rust

Puccinia leiocarpum (Syd.) Thirumalachar, Comb. nov.

Pycnia amphigenous, sub-hypodermal, orange yellow in colour, flask-shaped, with an ostiole and paraphyses. Aecia are amphigenous, cupulate, with well-developed peridia, peridial cells measuring $22 \times 17\,\mu$. Aeciospores are polyhydral, thin-walled, binucleate, smooth-walled with an indistinct germ pore. Aeciospores measure $17 \times 14\,\mu$. Telia are amphigenous, developing as warty excrescences, hypodermal, black in colour, two-celled and stipitate. Teliospores measure $25 \times 11\,\mu$ and the stipe measures $29\,\mu$. Teliospores germinate without a period of rest, and sporidia infect the same host.

Hab.—On living leaves of *Ocimum adscendens*, Bangalore.

Date of collection 20–11–1940.

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* Grateful thanks are due to Dr. K. M. Thomas, Mycologist, Agricultural Research Station, Coimbatore, for making available rust specimens for comparative studies.

Summary

1. The fungus attacks leaves and sometimes twigs. The infected portions appear as concentric blotches. Three spore forms, *viz.*, pycnia, æcia and telia are present.

2. Pycnia are subhypodermal-flask shaped and orange yellow in colour. The paraphyses are exserted from the ostiole.

3. Aecia are developed on both the surfaces of the leaves, and are covered with well-developed peridia. Aeciospores are polyhydral and smooth-walled. A single germ pore becomes visible during germination.

4. Telia are amphigenous and are black in colour. Teliospore is stipitate, and two-celled. Spores germinate without a period of rest. The basidiospores are thin-walled, and uninucleate. Some abnormal types of germinations have been described.

5. Basidiospores infect the same host and form pycnia and æcia, and thus it is manifest that the fungus is autœcious.

6. Spore measurements and other considerations goes to show that *Aecidium leiocarpum* Syd. is the æcial stage of *Puccinia leiocarpum* (Syd.) Thirumalachar. In conclusion the author wishes to acknowledge his indebtedness to Dr. M. A. Sampathkumaran, M.A., PH.D., Professor of Botany, Central College, Bangalore, for guidance and encouragement in the course of this work, and to Dr. B. B. Mundkur, Imperial Agricultural Research Institute, New Delhi, for the help given in identifying the species and valuable suggestions.

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Postscript

Since the paper was sent for publication, an account of *Puccinia Ocimi* Doidge, recorded by Doidge (*A Preliminary Study of the South African Rust Fungi, Bothalia* II, pp. 78, 1926) on *Ocimum suave* Willd. and *O. americanum* L., in South Africa, was made available by the kindness of Dr. B. B. Mundkur. It is claimed by Doidge that the teliospores observed is the perfect stage of *Aecidium Ocimi* first recorded by Hennings.

Comparative studies indicate that the teliospores of *Puccinia Ocimi* Doidge are very large in size compared with those of *Puccinia leiocarpum* (Syd.) Thirumalachar. The teliospores of *P. Ocimi* measure 40–60 × 16–24 μ, and those of *P. leiocarpum* only 25 × 11 μ. The pedicel of the teliospores of *P. Ocimi* are up to 80 μ in length, whereas in *P. leiocarpum* it is invariably 29 μ in length.

These differences in the measurements of the teliospores of *Puccinia Ocimi* and *P. leiocarpum*, taken up along with the differences in the size and structure of the æciospores, justify the consideration that *Puccinia leiocarpum* is a new species. It also supports Sydow's original conclusions for separating *Aecidium leiocarpum* Syd. from *A. Ocimi* P. Henn., and considering the former as a new species. Type specimen of *Puccinia leiocarpum* (Syd.) Thirumalachar Comb. nov., has been deposited in Herb. Crypt. Ind. Orient. of the Imperial Agricultural Research Institute, New Delhi.

CYTO-MORPHOLOGICAL FEATURES OF *PORTULACA TUBEROSA* ROXB.

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1. *Introduction*

The family Portulacaceæ has been studied to some extent by previous investigators. The earliest worker in this family is according to Schnarf (1931), Hofmeister (1858) who studied the embryology of *Calandrinia*. Later d'Hubert (1896) investigated two other genera, *viz.*, *Portulaca* and *Talinum*, in addition to *Calandrinia*. Rócen (1927) made a comparative study of the embryology and development of female gametophyte in about eight genera, belonging to this family including *Calandrinia*, *Claytonia*, *Montia*, *Portulaca*, and *Anacampseros*. In all these, he found the normal type of development.

Suguira (1936) gives a brief account of the previously determined chromosome numbers in this family. He has also reported the haploid numbers of *Calandrinia speciosa* and *C. umbellata* as twelve and ten respectively.

The genus *Portulaca* has also received some attention. Rócen (1927) studied the development of embryo-sac and embryo in *Portulaca grandiflora.* Later Cooper (1935 and 1940) studied the microsporogenesis and embryology of *Portulaca oleracea* whose haploid number, he reported as 27.

In this paper, the cytological and morphological features of *Portulaca tuberosa* Roxb., are described. Chromosome numbers of some of the local species of *Portulaca* are also given, having been determined for the first time.

2. *Materials and Methods*

Portulaca tuberosa Roxb., is a common perennial weed, with short stems spreading from a tuberous root. The root is 2–3 inches long and is slightly fusiform with a few branches towards the extremity. The leaves are arranged alternately on the spreading stem which is 2–3 inches long. The leaves are linear and fleshy with nodal appendages of sparingly tufted brown or silvery hairs. Flowers are in small clusters, surrounded by 7–10 leaves and tufted hairs and are yellow in colour. Stamens are many and the style is filiform and five-cleft.

Root-tips were collected from plants grown in pots and fixed in Müntzing's modification of Navaschin's fluid (Müntzing, 1933). For meiotic stages, whole buds were fixed with or without prefixation in Carnoy's fluid. Stages for morphological studies were obtained from small buds and ovaries fixed in formalin-acetic alcohol. The materials were embedded in paraffin wax using chloroform as the paraffin-solvent. Sections were taken at thicknesses varying from 6–15 microns and were stained in iron-alum hæmotoxylin and Newton's iodine Gentain-violet.

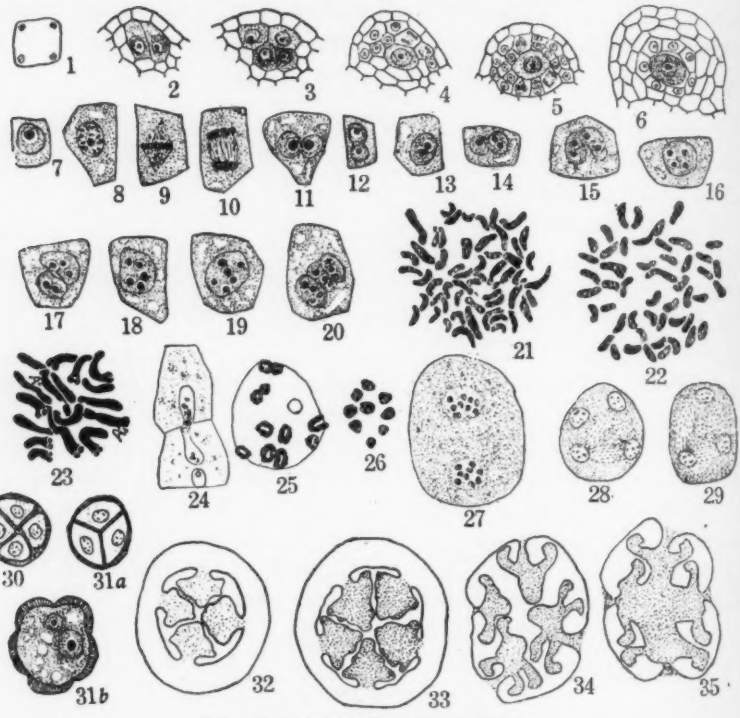
3. *Observations*

(*a*) *The microsporangium :*

The archesporium of the anther is differentiated at the four corners of the anther, when the four lobes have not been formed (Fig. 1). The archesporium consists of two cells which are hypodermal in position (Fig. 2). Cooper (1935) reports that a band of seven to eight cells constitute the archesporium in *Portulaca oleracea.* Archesporial bands such as are met with in the present case, have been observed in the genera *Bergia, Sesuvium* (Raghavan and Srinivasan, V. K. , 1940, *a* and *b*) and in *Spermacoce* (Raghavan and Srinivasan, A. R., 1941, *c*).

Through periclinal divisions of the archesporial cells, two layers are formed, the outer being the primary wall layer and the inner the primary sporogenous layer (Fig. 3). By further divisions of the primary wall layer, more parietal layers are formed surrounding the sporogenous cells (Figs. 4–5).

The sporogenous cells do not divide much and hence only one pollen mother cell is seen in each lobe of the anther in transverse sections. Presumably there is only one row of microsporogenous cells. We find this confirmed in longitudinal sections. At the mature stage, the wall of the anther consists of four layers, the innermost of which functions as the tapetum (Fig. 6).



TEXT-FIGS. 1–35. *Portulaca tuberosa*

FIGS. 1–35 represent stages of microsporogenesis and development of placenta. FIG. 1. Transverse sections of young anther. × 75. FIG. 2. Archesporium of the anther. × 750. FIG. 3. The primary wall layer and the primary sporogenous layer. × 750. FIGS. 4 and 5. Division of cells in wall layers forming more parietal layers. × 750. FIG. 6. Anther showing three wall layers and the tapetum. × 750. FIG. 7. Tapetal cell with a single nucleus. × 1500. FIGS. 8–10. Stages of division (mitotic) of the tapetal nuclei. × 1500. FIGS. 11–20. Various stages of fusion of tapetal nuclei. × 1500 (explanation in the text). FIG. 21. Somatic metaphase plate of *Portulaca* sp. × 3900. FIG. 22. Diploid chromosome complement of *Portulaca quadrifida*. × 3900. FIG. 23. Somatic plate of *Portulaca tuberosa*. × 3900. FIG. 24. Cytomixis in *P. tuberosa*. × 1100. FIG. 25. Diakinesis. × 3900. FIG. 26. Meiotic M I plate. × 3900. FIG. 27. M II plate. × 3900. FIGS. 28 and 29. Spindle formation in T II. × 1100. FIGS. 30 and 31 *a*. Isobilateral and tetrahedral tetrads. × 750. FIG. 31 *b*. Mature pollen grain. × 750. FIGS. 32 and 33. Early stages of placenta formation. × 150. FIG. 35. Placentas growing towards the centre. × 75. FIG. 35. Showing the central tissue formed by the fusion of the placentas. × 75.

The behaviour of the tapetal nuclei is worth mentioning. To begin with, the tapetal cells are uninucleate and are full of cytoplasm (Fig. 7). Later, small vacuoles arise in the cytoplasm and the nucleus shows signs of division (Fig. 8). The division of tapetal nucleus takes place even before the nuclei of the pollen mother cells enter into the early meiotic stages. Cooper (1935) observed that the division took place while the nuclei of the microspore mother cells were passing from synizesis into the heterotypic plate. The division in this case is mitotic as can be seen from Figs. 9–10. Some workers have reported amitosis as the typical method by which the tapetal nuclei divide. Such a case has been reported by O'Neil (1920) in *Datura stramonium*. Rócen (1927) also reports a similar amitotic division in *Portulaca grandiflora*. Cooper (1935), however, found that the tapetal nuclei in *Portulaca oleracea* multiply through ordinary mitotic division. These and other recent observations in many other genera distributed over a wide range of families can be taken as additional evidence to support the view that the nuclei of the tapetal cells divide mitotically. Cooper (1933) recognises three types of tapetal cells: (1) in which the tapetal cells remain uni-nucleate, (2) in which they are bi-nucleate and (3) where they become pluri-nucleate. The present case belongs to the third type and shows the interesting phenomenon of tapetal nuclear fusion. It is possible to trace through intermediary stages how multi-nucleolate nuclei arise. Fig. 11 shows a bi-nucleate tapetal cell and Fig. 12 represents the fusion of the nuclei. In Fig. 13, a tapetal cell with a two-nucleolated nucleus is shown. This is the result of the above said fusion. Fig. 14 represents the fusion of a bi-nucleolated nucleus with an ordinary nucleus to result in a three-nucleolated nucleus (Fig. 17). Two tapetal nuclei each having two nucleoli by fusing give rise to a four-nucleolated fusion nucleus (Figs. 15 & 16). The fusion of three-nucleolated nucleus and a two-nucleolated nucleus is shown in Fig. 17, which results in the formation of a five-nucleolated nucleus (Fig. 18). A peculiar case was found in which three nuclei were seen to fuse (Fig. 20). Of the three nuclei, two had three nucleoli and the remaining one, four nucleoli. Such a fusion will result in a nucleus having ten nucleoli. A completely fused ten-nucleolated body was, however, not met with. But as in Fig. 19, six-nucleolated fusion nucleus was not uncommon.

Fusion of tapetal nuclei resulting in the formation of multi-nucleolated nuclei have been met with in many cases. Such a phenomenon has been recorded in *Nicotiana glutinosa* (Raghavan and Srinivasan, A.R., 1941 *b*), in *Ranunculus* (Singh, 1936) and *Chenopodium* (Bhargava, 1936). It is interesting to note that Cooper (1935) found that in *Portulaca oleracea*, the tapetal cells remained bi-nucleate till their final degeneration. We are not told whether these fuse at all.

The pollen mother-cells round off and show starch grains in their cytoplasm. Such accumulation of starch grain in P.M.C.'s has been recorded in *Spathodea* (Raghavan and Venkatasubban, 1940).

(*b*) *Somatic chromosomes*:

The diploid chromosome number of this species is shown in the somatic plate in Fig. 23. There are 18 chromosomes of which one is found to have a satellite at its distal end connected by a trabant. Perhaps the body is too big to be called a satellite. It may be more appropriate to describe the chromosome as having a secondary constriction, the primary constriction being subterminal. Very likely the homologue of this chromosome (A) is A_1 where however the trabant is not so prominently seen owing to a possible twist and the orientation of the chromosome. Two of the chromosomes are comparatively short.

(*c*) *Meiosis*:

During early prophase stages the extrusion of chromatin material from one cell to its neighbouring pollen mother-cell, was observed in some cases. Such extrusion is commonly known as cytomixis. In this case, the prophase threads formed a sort of wad and this chromatin lump was extruded along with the nucleolus into the adjacent cell (Fig. 24). Such a phenomenon has been met with in the interspecific hybrid between *Nicotiana tabacum* × *N. glutinosa* and its importance has already been discussed (Raghavan and Srinivasan, A. R., 1941 *a*).

Cytomixis generally is the cause of meiotic abnormalities, obviously due to a disturbance of the chromosome balance in the pollen mother-cells. Though the phenomenon occurred here and there, not many abnormalities worth recounting were met with, so far as the meiotic process is concerned.

During diakinesis nine bivalents are formed (Fig. 25). The bivalents are fairly large. Two of the bivalents possess three chiasmata, two terminal and one interstitial. Six ordinary ring bivalents are met with, each with two terminal chiasmata. Only one bivalent showed a single terminal chiasma, which is obviously the result of the pairing of the two shortest chromosomes. The nuclear membrane disappears and the nucleus after a short prometaphase enters upon the metaphase. During metaphase, nine bivalents are observed, all arranged in the equatorial plate (Fig. 26). Disjunction seems to be normal and at second metaphase nine chromosomes are seen at each of the poles (Fig. 27).

During second telophase (Figs. 28 & 29) four groups of chromosomes are organised and six spindles are formed connecting each of the telophase

groups with the other three. When all the four groups are arranged in one and the same plane (Fig. 28), there is an isobilateral arrangement which after the process of furrowing gives rise to an isobilateral tetrad (Fig. 30). If however, the telophase groups are situated at right angles to one another, the tetrahedral type is the result (Figs. 29 & 31*a*). The furrowing takes place simultaneously. The furrows formed are very narrow during initial stages and the process is apt to be construed as cell-plate formation. This however is not the case in the present investigation, for, not only has actual furrowing been observed, but other structural details necessary for cell-plate formation are not present also. For instance, if organisation of the tetrads was by cell-plate formation, then a definite thickened portion formed by the aggregation of small particles of cell wall matter must be observable in the middle of each spindle. The spindle itself should have been greatly stretched laterally. These were not observed in the present case.

Healthy pollen grains of large size are formed which are two celled at the shedding time (Fig. 31 *b*). Cooper (1935) reports that the pollen grains are three-nucleate at the shedding time.

(*d*) *Development of the placenta and the ovules :*

The ovary is unilocular and half inferior. It is made up of two to five carpels. A somewhat critical ontogenetic study of the ovary reveals some interesting facts. In the earliest recognisable stage the gynæceum, formed by the marginal fusion of the four carpels (Figs. 32 & 33), shows parietal placentation. The primordia of the ovules look like small knob-like protuberances. At a later stage the tips of these carpels namely, the non-ovule bearing conical tips of the placenta, come together and fuse; so much so, a structure that may be regarded as axile placenta is formed (Figs. 34 & 35). After this the placentas gradually lose their connection with the ovary wall so that the ovule seems to arise from placental structures which are free from the surrounding wall of the ovary. Strictly speaking the wall of the ovary is composed of the four or five carpels which are fused together to form a tubular structure. Internal to this there is this placental region bearing the ovules. This is a condition which simulates a free-central placentation but which cannot be strictly regarded as such, as it would mean an ovule bearing tissue taking its origin from the base of the ovary. The points of interest that may be raised in passing are (1) whether this is truly a case of free-central placentation, and (2) if that is so can the developmental history of the placenta throw any light upon its derivation. If the recapitulation theory is applicable in this case, then we can say that the order of development is parietal, axile and free central. A generalisation on this matter must however await

a more critical and widespread ontogenetic study of the members especially of the Centrospermeæ.

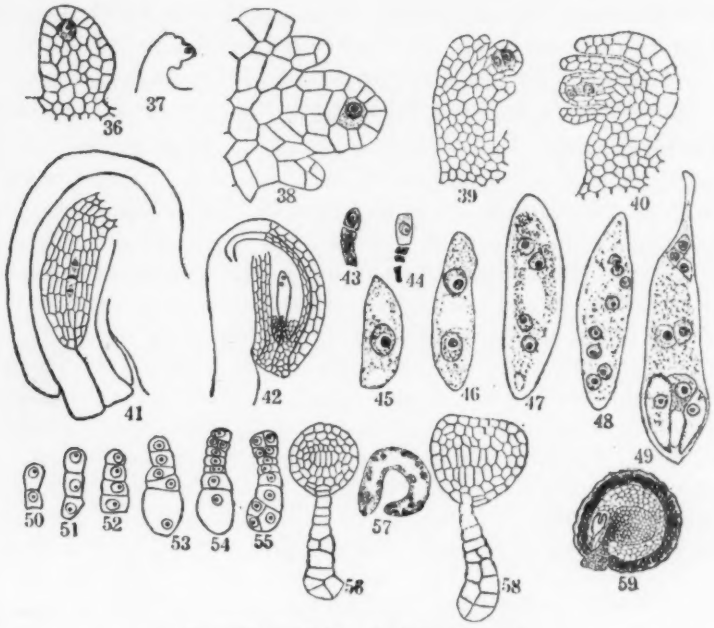
The archesporium of the ovule is differentiated when the ovule arises as a straight protuberance from the placenta (Fig. 36). It consists of a single hypodermal cell larger than the surrounding cells rich in cytoplasm and having a prominent nucleus. During the growth of the ovules, the funicular portion elongates and the body of the ovule bends towards the one side (Fig. 37). Later the archesporium divides periclinally to result in the primary parietal cell lying on the outside and the primary sporogenous cell inside (Fig. 39). The primary sporogenous cell without further division elongates and functions as the megaspore mother-cell and is invested by two layers of nucellus on its side, capped by the primary parietal cell at the top (Fig. 40). The primary parietal cell gives rise to three or four layers of wall cells so that at later stages the dyad and the embryo-sac come to be situated three or four layers deep in the nucellus (Figs. 41 & 42). The nucellar cells at the sides of the megaspore mother-cell also divide causing the ovules to become massive. During the early embryo-sac stages the ovule is anatropus (Fig. 42). But later it bends on itself to give rise to the curved embryo-sac and thus forms a campylotropous structure. In the mature ovule (Fig. 59) the ovule becomes so curved that the micropylar and the chalazal ends nearly meet and most of the funicular tissue is caught in between them.

The two integuments make their appearance when the ovule begins to curve towards one side (Fig. 37). The inner integument is the first to make its appearance (Fig. 38). It is at first two layers thick (Figs. 38–40). It continues to remain so during later stages also, except at the micropylar region where it is three or four layers thick (Fig. 42). The inner integumental halves meet at the micropylar region and constitute the micropyle. The outer integument is differentiated soon after the inner integument. But that half of the outer integument on the side of the ovule towards the funicle is not differentiated until late in the development of the megaspore mother-cell (Figs. 39–41).

The outer integument even at later stages does not overtake the inner integument and therefore cannot be said to contribute to the organisation of the micropyle. It remains two layered throughout.

(*e*) *Megasporogenesis :*

The nucleus of the megaspore mother-cell undergoes meiosis and results in the dyad. The cells of the dyad are more or less of equal size (Fig. 41). Cooper (1940) however has found in *Portulaca oleracea* that the chalazal cell is larger and increases in size during interkinesis. He has also reported that the spindle in the micropylar cell may be at any angle. In this species,



TEXT.-FIGS. 36–59. *Portulaca tuberosa*

FIGS. 36–59 show the development of embryo-sac and embryo. FIG. 36. Archesporium of the ovule. × 750. FIG. 37. The ovule at a later stage. × 75. FIG. 38. The same magnified showing the development of integuments. × 1500. FIG. 39. Primary parietal and primary sporogenous cells. × 750. FIG. 40. Megaspore mother-cell. × 750. FIG. 41. Ovule at the dyad stage. × 750. FIG. 42. Ovules at the 4-nucleate embryo-sac stages. × 350. FIGS. 43 and 44. Linear tetrads. × 750. FIGS. 45–47. Embryo-sac stages. Explanation in the text. × 1500. FIG. 48. Eight-nucleate embryo-sac at an early stage. × 1100. FIG. 49. Mature embryo-sac. × 1500. FIGS. 50–56. Embryo stages. × 750. FIG. 57. Shows the nuclear endosperm. × 75. FIG. 58. Embryo just before lobing of cotyledons. × 750. FIG. 59. Section of a young seed. × 75.

however, such is not the case. Even during homotypic division the dyad cells are of equal size and both the spindles are in a straight line (Fig. 41). Cooper (1940) adds that the division in the micropylar cell lags behind that of the chalazal cell. In the present case we find simultaneous division resulting in a linear tetrad (Fig. 43) of megaspores. The chalazal megaspore develops while the other three degenerate (Fig. 44). The megaspore develops into the uni-nucleate embryo-sac (Fig. 45). The nucleus divides and the daughter nuclei travel to the opposite poles and the two-nucleate embryo-sac is formed (Fig. 46). Another division of the embryo-sac nuclei results in the formation of the four-nucleate embryo-sac (Fig. 47). Starch grains are formed at this stage in the embryo-sac and the cytoplasm is very vacuolate. The four

nuclei divide to give rise to eight-nucleate embryo-sac. Of the eight nuclei, one from each pole travels towards the centre to fuse later into the secondary nucleus (Fig. 49). Thus there are three nuclei left at each of the poles. The three nuclei at the micropylar end are bigger than those at the chalazal end.

The mature embryo-sac is long and tapering at the antipodal end (Fig. 49). It consists of the egg-apparatus at the micropylar end, the antipodals at the chalazal end and the polar nuclei in the centre. The egg-apparatus consists of the synergid cells, the ends of which lie apart from each other during early stages but come closer during later stages. The synergids are large, each with a basal vacuole and the nucleus above the vacuole. No starch grains were found in the synergids. Cooper (1940) has found a distinct filiform apparatus in the synergids of *Portulaca oleracea*. The egg nucleus is much larger than the nuclei either of the synergids or of the antipodals. The egg cell has a large apical vacuole and its cytoplasm contains inclusions of starch grains. The antipodal cells are three in number and are small in size. They are arranged in the form of the letter V. In *Portulaca oleracea* they were found to be located in an axial row at the chalazal end. These are ephemeral and degenerate before fertilization.

(*f*) *Endosperm and embryo* :

After fertilization, the endosperm nucleus divides and gives rise to free nuclear endosperm. The endosperm remains nuclear until a spherical mass of embryo cell is formed (Fig. 57). Cell-wall formation takes place only later resulting in loose cells.

The oospore undergoes a transverse division followed by cell-wall formation. Thus a two-celled pro-embryo is formed (Fig. 50). Through further divisions the three, four and five-celled pro-embryos are formed (Figs. 51–53). The basal cell and the cell next to that increase in size (Figs. 53 & 54). After a linear pro-embryo of seven cells is formed, the cell next to the apical cell undergoes an anticlinal division giving rise to the quadrant (Fig. 54). Thus the embryo at this stage consists of the quadrant and a uniseriate suspensor five cells long. The cell of the suspensor next to the quadrant becomes the hypophysis, while the quadrant through the anticlinal division of the apical cell, results in the formation of an octant (Fig. 55). At this stage the lowermost cell of the suspensor undergoes a periclinal division resulting in a small basal cell and a large apical cell, the latter of which divides anticlinally. Thus the basal cell of the suspensor becomes biseriate during the octant stages.

Periclinal and anticlinal walls are laid in the octant and a spherical mass of cells is formed (Fig. 56). Anticlinal divisions in the basal suspensor cells

take place so that at a later stage each of the five basal rows of suspensor cells come to be made up of two cells (Fig. 58).

Fig. 59 shows a young seed in which the cotyledonary lobing of the embryo has been effected. The endosperm is cellular and soon the embryo eats up the whole endosperm. The mature seed is thus exalbuminous. The testa consists of two layers of which the inner layer is sclerotic.

(*g*) *Some chromosome numbers in the genus,* Portulaca *:*

Portulaca sp.—54 Chromosomes were counted in somatic metaphase plates (Fig. 21). Many of the chromosomes are short. Two of the medium-sized chromosomes show satellites.

Portulaca quadrifida Linn. (Fig. 22).—Sections of root-tips show metaphase plates with forty-eight chromosomes. All the chromosomes except two are short.

4. *Discussion*

(*a*) *The behaviour of tapetal nuclei*; *its possible meaning*:

In a number of genera investigated in this laboratory, it has been found that the division of the nuclei of the tapetum (of the microsporangium) and their ultimate fusion prior to disintegration is almost the rule. The real significance of this repeated division of the tapetal nuclei and their later fusion is not quite clear. That the cells of the tapetum are nutritive in function, there can be no doubt. If this is conceded, we have to interpret the division and fusion of the nuclei only on the basis of this primary nutritive rôle. Possibly, an analogy can be drawn from that important nutritive tissue in angiosperms, the endosperm, where it is the result of repeated division of the triple fusion nucleus. First of all there is a fusion of three nuclei, two polars and the male, and then a subsequent division, either into free-nuclei or a cellular structure, to form the so-called endosperm tissue. It may be that in the tapetal cells, nutritive as they primarily are, a sort of a fusion of nuclei must precede the formation of nutritive materials ; hence the nuclei divide. This not only increases nuclear material, *i.e.*, the nutritive material, but also acts as a stimulus even as in the case of the triple fusion nucleus. The tapetal nuclear behaviour may be regarded in some measure as a simulation of the triple fusion nucleus. Seldom do we find the divided nuclei in a separate condition. In other words, immediately upon their division they fuse. While it is likely as has been mentioned above, that this fusion is a simulation of the sexual act which precedes the formation of the other nutritive tissue, it is likely that it is also governed by spatial considerations. Obviously, the fusion product of four or five nuclei occupies far less space than five separate nuclei, hence perhaps this immediate fusion.

(*b*) *Morphological details*; *are there any too minor to recount*?

In the course of cyto-morphological investigations upon different plants, there is a wide measure of uniformity so far as broad details of micro- and macro-sporogeneses are concerned. The question is pertinently asked, whether there is any need to recount all these details in different genera though they may belong to different widely separated families. In essence the morphological details are the same; why then should one almost repeat these details in various genera? The answer for this question must be found in another question; are these morphological details (pertaining to the ontogeny of the micro- and mega-gametophytes) of any value taxonomically and phylogenetically? or, are they merely descriptive and devoid of any such significance? To our mind the former seems to be more likely.

It is interesting that what may be regarded as important features are not characteristic even of a genus, not to speak of a family. While in the course of investigation we find some features characterising almost all the members of a particular genus and even members of the family, we find at the same time that, species belonging to the same genus show wide variations in respect of features which at first sight may be regarded as important. So far as *Portulaca* is concerned, the present species *P. tuberosa* differs from the previously investigated *P. oleracea* in some important details. For instance, in *P. oleracea* only bi-nucleate tapetal cells were found, whereas in the present species multinucleate tapetal cells are the rule. In *P. grandiflora* multinucleate tapetal cells were met with. The condition of the microspore in the present species and *P. grandiflora* is two-nucleate at the time of shedding whereas it is three-nucleate in *P. oleracea*. It only shows that so far as these morphological details are concerned there cannot be any type feature that could be said to be characteristic either of the genus or of the family. That could be found out only by a critical examination of a large number of species belonging to the genus or of a number of genera comprising a family. If at the end of such a study we find some features common throughout, then only can it be said that they are characteristic. It might well be that what may at first sight be regarded as insignificant details may be of considerable importance from this point of view. An instance of this kind may be drawn from the critical studies of Aizoaceæ (Raghavan and Srinivasan, V. K., 1940 *b*); it was found that the two tribes of the Aizoaceæ, the Molluginoideæ and the Ficoideæ, were characterized by a number of morphological features, some of which like the presence of the aril and its absence may rightly be considered as important morphological characters. But alongside of this, such minor features as the radial elongation of the epidermal cells of the

nucellus, the number of layers constituting the integument, etc., were also found to be useful in determining the characteristic features of the two sub-groups. Another instance of this kind can be had in the investigations in the family Elatinaceæ (Raghavan and Srinivasan, V. K., 1940 *a*) where, morphological details such as the order of wall formation in the endosperm tissue have been made use of to support the division of Elatinaceæ into two sub-groups, the Elatinoideæ and the Bergioideæ, which division has been made, on the basis of chromosome numbers and other floral characters. Similarly, in the Rubiaceæ (Raghavan and Srinivasan, A. R., 1941 *c*) such apparently insignificant details as the number of cells composing the nucellar epidermis, the configuration of the epidermis, etc., which might well be passed over in a casual study, were upon a critical examination, found to play an important rôle in the matter of the evolution of chromosome numbers in the family. All these only go to show that however minor and insignificant any morphological detail may be from a superficial point of view, it may be of significance for purposes of generalization which, however, means a more critical and wide study of a particular group. In other words no morphological detail is too insignificant to be recorded. On the other hand important characters may have no morphological value in a general critical study either of a genus or of a family.

(*c*) *The position of Portulacaceæ :*

A word may now be said of the position of Portulacaceæ about which there is difference of opinion. Taxonomists have assigned to it one position or another based almost solely upon floral characters and some vegetative features. A few like Puri and Singh (1935) have assigned to it another position mainly upon the basis of anatomical features. No one has so far tackled this problem from the point of view of chromosome numbers. As cyto-taxanomy is coming to play an increasingly important part, it would be well worth our while to review the position of the *Portulacaceæ* from this point of view also and try if possible, to correlate the position indicated by chromosome numbers, with other data on the basis of which the taxonomical position of this family has so far been determined.

Bentham and Hooker (1862–1883) have grouped *Portulacaceæ* under *Thalamifloreæ* along with *Caryophyllaceæ* (the other minor families like *Frankeniaceæ*, etc., are not mentioned as they are not necessary for our purpose). Engler and Prantl (1894) placed this family in the *Monochlamydeæ* under the group *Centrospermeæ* along with seven other families showing curved embryos. Hutchinson (1926) however divides the *Centrospermeæ* into two groups, the *Chenopodiales* and the *Caryophyllales*. *Portulacaceæ*,

Caryophyllaceæ and *Aizoaceæ* come under *Caryophyllales* while the rest are grouped under *Chenopodiales*.

Puri and Singh (1935) are of opinion that the families, *Chenopodiaceæ*, *Amaranthaceæ*, *Nyctaginaceæ*, *Phytolacaceæ* and *Aizoaceæ* form a very natural grouping. That is, they separate *Aizoaceæ* from *Portulacaceæ*. It differs from Hutchinson's classification in this important feature. In effect their classification is very like that of Bentham and Hooker (1862–1883), though different names are given to the two groups (*Centrospermales* = *Apetalæ* of Hooker and *Caryophyllales* = the *Polypetalæ* of Hooker). Their opinion is based on some embryological details for which phylogenetic importance has been assigned. They also consider anomalous secondary thickening a very important anatomical character, which being present in *Aizoaceæ*, is the reason for its removal from the *Caryophyllales* of Hutchinson to the Centrospermales where it will fit in better with the other orders showing anomalous secondary thickening. The different systems of classification pertaining to this are roughly summarisd in the accompanying table. Chromosome numbers in the different families are also indicated.

On closer scrutiny it seems that the affinities of *Portulacaceæ* have not been given due consideration, when forming the latter said classification. The external features of *Portulacaceæ* bear a great resemblance to those of *Aizoaceæ*. In habit, both are essentially herbaceous. Both include genera with succulent vegetative parts, circumcessile capsules and above all, curved embryo. The embryological features that these authors report to be characteristic of the families in the *Centrospermales*, are found to occur in *Portulacaceæ* also, with the exception of one or two, *e.g.*, the absence of anomalous secondary thickening, the non-occurrence of periclinal divisions in the nucellar epidermis, and the two-nucleate conditon of pollen in *Portulacaceæ*.

Table representing the position of Portulacaceæ according to various Systems of Classifications

[Haploid chromosome numbers occurring in the families are quoted from Tischler's list of chromosome numbers (Tischler, 1938)]

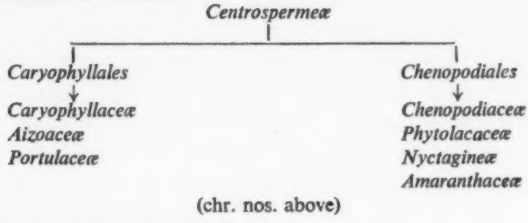
(1) According to Bentham and Hooker (1862–83):

Thalamifloreæ —— *Caryophyllineæ* —— *Caryophyllaceæ*, n = 8, 9, 10, 11, 12, 13, 15, 30 & 45.
Portulacaceæ n = 8, 9, 10, 18, 24 & 27.

(2) According to Engler and Prantl (1894). Rendle (1938) has in the main followed this classification:

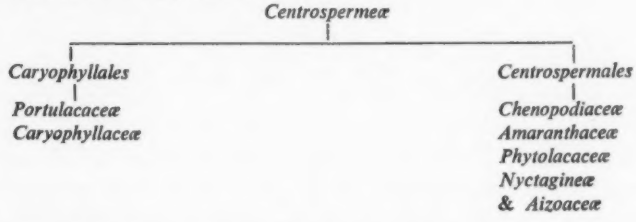
Monochlamydeæ
|
Centrospermeæ
|
Chenopodiaceæ, n = 9 & 18; *Amaranthaceæ*, n = 6 & 21; *Nyctagineæ*, n = 29; *Phytolaccaceæ*, n = 9, 18, 36 & 54; *Aizoaceæ*, n=9, 16 & 32; *Portulacaceæ* (chr. nos. given above); *Caryophyllaceæ* (chr. nos. above).

(3) According to Hutchinson (1926):



(chr. nos. above)

(4) According to Puri and Singh (1935):



Examined in the light of chromosome numbers, two features are found to be most striking, first, the relationship betweem *Portulacaceæ* and the *Centrospermales* of Puri and Singh, and secondly the relative lack of similarity of the chromosome numbers of *Portulacaceæ* and those of the *Caryophyllaceæ*. Considering the first-mentioned feature, we find that the chromosome numbers of *Portulacaceæ* mainly belong to the three and four series. Suguira (1936) after considering the chromosome numbers in the genus *Portulaca* has come to the conclusion that nine is the basic number of the genus. The same author in a later work (Suguira, 1940) observes, "According to Pax, *Portulacaceæ* and *Aizoaceæ* are closely related. Karyologically this can be recognized too, for they both have a theoretical basic number of three." Perhaps from this primary basic number 3, a secondary basic number 4 also arose, of which we get multiples as representatives. Examining the chromosome numbers of the *Centrospermales* in this light, we find that the chromosome numbers of *Aizoaceæ* are multiples of 3 and 4, those of *Phytolaccaceæ*, *Amaranthaceæ* and *Chenopodiaceæ*, multiples of 3 only. *Nyctagineæ* alone shows 29 as the chromosome number of *Mirabilis*. Thus from the point of view of chromosome numbers *Portulacaceæ* seems to be closely related to the members of the *Centrospermales* of Puri and Singh (1935).

Secondly, the grouping of *Portulacaceæ* along with *Caryophyllaceæ* may be considered. Viewed in the light of chromosome numbers, this grouping seems to be unnatural. *Caryophyllaceæ* has been pointed out to be a large and heterogenous family. Puri and Singh (1935) have said that the

largeness and the variety in its structure warrant the elevation of the family to an independent order. This is supported by the nature of the chromosome numbers also. There is a gradation of chromosome numbers from 8 to 13. The majority of the numbers occurring in the polyploid series are multiples of 5 and not of 3 or 4. Thus from the point of view of chromosome numbers the *Caryophyllaceæ* is less related to *Portulacaceæ*, than the families included in the *Centrospermales* are to the latter.

It thus seems to us, that there is not much justification for separating *Portulacaceæ* and *Aizoaceæ*. They conform to one another, not only in chromosome numbers but also in other important details. It would therefore be more in accordance with the data available so far, including chromosome numbers, to remove *Portulacaceæ* from the *Caryophyllales* of Puri and Singh and bring it under the other group along with *Aizoaceæ*. This would mean that *Caryophyllaceæ* would form a group by itself and the rest of the families would constitute the other group. Some data which Puri and Singh (1935) think important for the separation of *Aizoaceæ* from the *Portulacaceæ* are now disclosed by the present investigation to be of no great morphological value. For example, *Aizoaceæ* has three-celled pollen grain while *Portulacaceæ* has only two-celled pollen, and hence it is contended that they cannot be put together. But it has been shown in the present investigation that this is a feature which varies among the members of even a single genus and therefore this cannot be given much importance.

The other alternative is to retain Hutchinson's classification, which would statisfy the important condition of keeping *Portulacaceæ* and *Aizoaceæ* together. But along with these, *Caryophyllaceæ* also would occur. Sufficient data relating to the affinities between *Aizoaceæ* and *Portulacaceæ* on the one hand, and *Caryophyllaceæ* on the other, not being available, we consider that inclusion of *Portulacaceæ* in the *Centrospermales* and the isolation of *Caryophyllaceæ* is not unjustified.

The classification modified on the above said lines would be as follows:

Centrospermæ

Centrospermales	Chr. nos.	*Caryophyllales*	Chr. nos.
Chenopodiaceæ	3-series	*Caryophyllaceæ*	Aneuploid series from 8–13 and polyploid nos. in the 5-series
Amaranthaceæ	do.		
Nyctagineæ	29		
Phytolaccaceæ	3-series		
Aizoaceæ	3 & 4 series		
Portulacaceæ	do.		

5. Summary

The development of the microsporangium of *Portulaca tuberosa*, Roxb., is described. Multinucleate tapetal cells and tapetal nuclear fusion are met with.

The diploid number of chromosomes was found to be 18, which was confirmed by the meiotic counts. Diploid chromosome numbers of two other species of *Portulaca* are also recorded for the first time.

Meiosis is normal and the microspore is two-celled at the shedding time.

Development of the ovary and the ovules is described in some detail. Embryo-sac formation and embryo development are of the normal type.

The possible meaning of the tapetal nuclear fusion is discussed from the view-point of the nutritive function of the tapetal cells.

The importance of recounting morphological details in spite of their repetition, is discussed in the light of observations recorded in this paper. The position of *Portulacaceæ* is considered cyto-taxonomically.

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ERRATA

Volume XIV, No. 4, Section B

Pages 341–58 and Plates XVI-XVIII top *for* "Sunder Raj" *read* "Sundara Raj".

Page 342, line 1 *for* "investigation" *read* "investigations".

Page 344, line 27 *for* "hearsey" *read* "hearsay".

Page 350, line 7 *for* "are" *read* "is".

Page 350, line 30 *for* "fourteen" *read* "twenty-four".

Page 351, footnote *for* "Sundar" *read* "Sundara".

Page 356, footnote line 15 *for* "Horo" *read* "Hora".

Page 357, line 18 *for* "spwan" *read* "spawn".

Page 357, footnote *for* "101" *read* "106".

Plate XVI *for* "Barbus tor" *read* "Barbus tor H.B."

INDIAN SILICIFIED PLANTS

I. *Azolla intertrappea* Sah. & H. S. Rao

(With one Map and three Plates)

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Received September 27, 1941

Introduction

RECENT discoveries in this country of localities where petrified plants showing structure are to be found in abundance, have opened up a vast field for research in the silicified floras of India. Silicified plants, particularly fossil woods, have long been known from parts of India and Burma, but it was only in recent years that localities were found where a whole stratum is sometimes seen packed with well preserved petrifactions representing a rich and varied flora. The investigation of these plants in thin sections has already yielded results of palæobotanical as well as geological interest (Sahni, 1938, pp. 42, 58) and promises a rich harvest of results for the future.

Two areas deserve special mention here. The first is the Jurassic terrain of the Rajmahal Hills, embraced by the great loop of the Ganges east of Bhagalpur, in Behar. The second is the volcanic plateau of the Deccan which, like the Rajmahal area, is dotted over with fossiliferous localities, particularly in the Nagpur and Chhindwara districts. Both in Behar and in the Deccan the fossils are preserved in lenticles of fresh-water sediments interbedded with volcanic lavas which must have been poured out intermittently. The fine state of preservation of the plants in both the areas is probably due to their having been suddenly overwhelmed by showers of volcanic ash or by the acid lava flowing rapidly over the lakes and rivers, thereby sealing up the organic remains and preventing their transport to any distance before they became petrified, the lava or ash contributing the silica for the replacement of the tissues.

In the richness of the flora and in the quality of preservation these deposits are not inferior to the classical coal-balls of Europe, but as the plants are silicified their investigation cannot proceed so fast as in the case of the calcified remains of the northern coal measures. A nearer parallel

is seen in the plant-bearing cherts of Central France and in the buried petrified forests of Saxony which have yielded such remarkable results at the hands of numerous investigators since the time of Bernhard von Cotta.

The age of the Rajmahal beds is unquestionably Jurassic. It was never disputed until a few years ago when Dr. L. F. Spath (1933), on very indirect and scarcely admissible grounds, suggested that it may be Lower Cretaceous. This idea seems to have received some support from Dr. G. de P. Cotter of the Geological Survey of India, who has erroneously attributed this view to me (Cotter, 1940, 88): but the Rajmahal flora is now too well known to be taken for anything but a typical Jurassic flora.

On the other hand, the age of the Deccan lavas has been a hot-bed of discussion (Sahni, 1934, 134–36; 1937, 459–64; 1938). These basalts, like the fossiliferous intercalations known as the Intertrappean Beds, were originally classed as early Tertiary; then they were for many years regarded by the Geological Survey as Late Cretaceous, largely under the influence of W. T. Blanford's work. This view the writer also shared for several years. Smith Woodward's evidence (1908) that some fossil fishes indicated a Tertiary age was not taken seriously: down to the year 1937 the Cretaceous view prevailed with most geologists in India, although it was sometimes admitted that the eruptions may have continued into the Tertiary. At the present time the traps are again generally accepted as Tertiary, even for the crucial area of the Nagpur and Chhindwara districts where some of the oldest traps are seen directly in contact with the Archaean gneiss.

This revival of the Tertiary view dates from the year 1933 when the present writer (see Sahni, Srivastava and Rao, 1934, postscript, p. 27), reviewing the Deccan flora as a whole, was impressed by its general Tertiary aspect, and by the discovery of the modern genera *Nipa* and *Azolla* in this flora. As this evidence came from the Nagpur-Chhindwara region the grounds for assigning a Tertiary age to the traps were particularly strong (Sahni, 1934; 1937; 1938, 59–65). Since then much other evidence from various directions has converged to the same conclusion, with the result that we may now regard this as the view generally accepted (Sahni, 1940).

Collections of silicified plants from the Rajmahal Hills, the Deccan and other parts of India have now accumulated and will continue to increase much faster than the speed at which they can be described, unless teamwork is organised to tackle the material. For this purpose a working committee of Indian palæobotanists was recently formed. An annual progress report of Palæobotany in India is published in *The Journal of the Indian*

Botanical Society, and we are at least able to record the main discoveries and indicate the lines on which work is being carried on.

It was at first thought that the work on the two most important silicified floras of India, namely, those of the Deccan and the Rajmahal Hills, might be published in two comprehensive monographs. But the continued accession of fresh material makes such a plan impracticable. It is now proposed that as the detailed investigation of these plant remains proceeds full descriptions of them should be published from time to time, as they become ready. It is inevitable that sometimes, even after the available material of a species has been described, further and better material of it will be discovered. It will then be necessary to revert to the same subject later. The following account of *Azolla intertrappea* is a case in point. In a joint paper with Dr. H. S. Rao, sent for publication last year as a memoir in the *Palæontologia Indica*, this species was described in considerable detail, but the discovery of the unusually interesting specimen described below has now materially added to our knowledge of this plant.

1

Azolla intertrappea Sahni and H. S. Rao

1931 *Azolla* sp., *Rec. Geol. Surv. Ind.*, 65 (i), 22.

1934 *Azolla* sp., *ibid.*, 68 (i), 22–23.

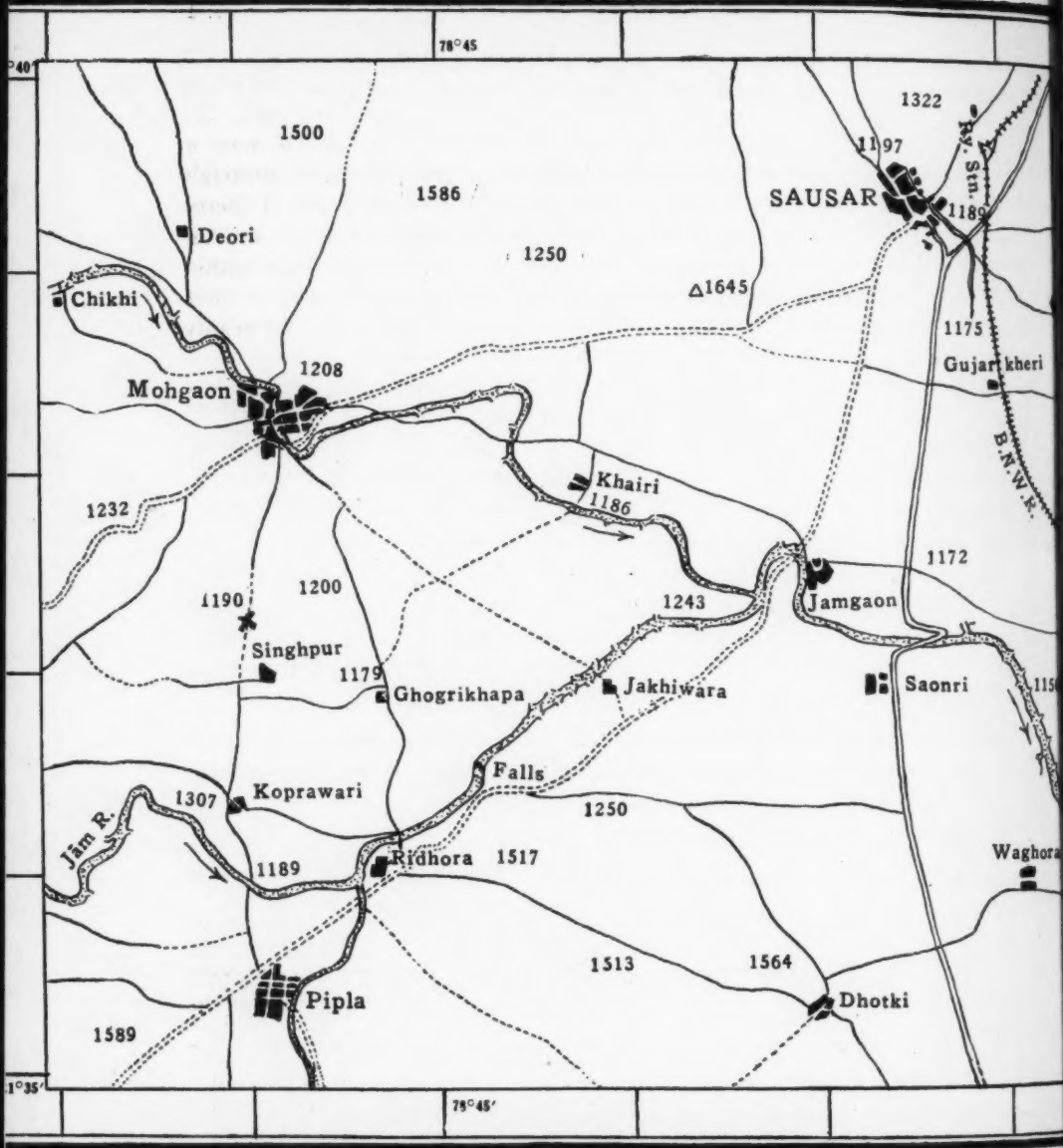
1934 *Azolla intertrappea* Sahni and Rao in Sahni, Srivastava and Rao, 1935, 26–27.

1938 *Azolla* sp. in Sahni, 1938, 66.

1940 *Azolla intertrappea* in Sahni, 1940, 14, Pl. 2, Figs. 7–9.

Of all the beautifully preserved plant remains to be found in the cherts round Sausar in the district of Chhindwara (see map), one of the commonest species is *Azolla intertrappea* (Sahni and Rao, 1934, 26–27). As I have said elsewhere, there is little doubt that these cherts represent silicified lake muds, sometimes mixed with volcanic ash deposited on stagnant waters.

The first evidence of this extinct water-fern, which I believe is geologically the oldest known representative of this familiar modern genus, was provided by a small block of chert sent me for examination by the Director of the Geological Survey in the year 1925. It was a solitary massula of roughly triangular shape, with several unmistakable anchor-tipped glochidia projecting from a frothy pseudocellular mass in which a number of darker bodies,



Map of the vicinity of Sausar, C. P.

Scale 1 inch = 1 mile.

(Simplified from the Survey of India sheets 55 k/10 & 55 k/14.)

Main roads, metalled — Cart track

" " unmetalled ---- Cattle track

Railway

Figures indicate elevation in feet.

undoubtedly the microspores, were embedded. A reference to this discovery was made in a brief note to the Director of the Survey and announced in his Reports for the years 1930 and 1933 (*Rec. Geol. Surv. Ind.*, **65**, 22; **68**, 22–23). Associated with this isolated massula of *Azolla* was a wealth of material of other interesting plant types which, although extremely fragmentary, were in a fine state of preservation. I therefore took an early opportunity of paying a visit to Sausar, and on New Year's Day, 1926, I collected from the fields and hill slopes within a radius of a few miles round that town a number of loose blocks of chert which on being sectioned yielded a flora of surprising richness and beauty of preservation.

It was evident that there was in these cherts a rich palæobotanical museum containing a silicified flora of great variety and interest such as had never yet been suspected in this country, and is certainly unparalleled in any other part of the world if we confine our comparison to floras of a similar age.*

Since the excursion to Sausar I have collected plant remains at several other localities in the Chhindwara District, especially at Mohgaon Kalan, a locality first discovered by Professor K. P. Rode and visited several times since by others. I have seen the same species of *Azolla* occurring abundantly at Mohgaon Kalan, where also have been collected other members of a rich flora not less interesting than that of Sausar.

It may be added that the discovery of an undoubted species of *Azolla* in these basal intertrappeans of Sausar and Mohgaon Kalan has taken a significant share in the successful attack on the Cretaceous theory of the Deccan Traps, in which I once myself believed.

Description

In the joint paper with Dr. Rao, now in the press, the leaf, root, rhizome, microsporangia and massulæ as well as the megasporocarp were described and figured. The diagnosis of the species as given there is as follows:—

Diagnosis.—Leaves about 1·3 mm. long by about 1 mm. broad, strongly convex, deltoid-pyramidal, with a finely papillate surface. Stomata, stem

* I have good reason to believe that an organised search in the inter-basaltic fresh-water deposits of N.W. Britain and N. America will yield silicified plant remains of perhaps equal beauty and variety. Similar hopes may be expressed concerning the intercalations of fresh-water deposits within the volcanic series in Abyssinia, East Africa and South Africa, which ought to be subjected to a thorough examination in thin sections.

and root anatomy similar to that of modern species. Microsporangia spherical, 205 μ in average diameter, each with several sector-shaped massulæ armed with stiff, anchor-tipped glochidia all round. Megasporocarp ovoid to broadly ovoid, 386–424 μ long $\times$ 250–295 μ broad; floats probably three, forming a deep helmet over the megaspore, covering the latter down to about half its height; megaspore spherical, about 215 μ in diameter, sending a broadly pyramidal process 136 $\times$ 76 μ into the base of the float mass.

This diagnosis now needs a slight amendment in the light of the present specimen.

Figured specimen.—Sahni Collection.

Locality.—Singhpur, Sausar Tehsil, Chhindwara District, C.P.; in a block of chert not found *in situ*, but undoubtedly washed out of an Intertrappean bed. The $\times$ near Singhpur on the map marks a prolific *Azolla* locality.

Horizon.—Deccan Intertrappean Series (Lower part of the Deccan Trap Series).

Age.—Early Tertiary, probably Eocene.

When describing the earlier material the constant presence of anchor-tipped glochidia on the massulæ suggested that a search should be made for the fine fibrils on the megasporocarp to which, in living species of the genus, the massulæ are known to become anchored. A careful examination revealed faint but unmistakable fibrils in the expected position in a single megasporocarp, which was figured.

The specimen now discovered is remarkable, among other things, for the fact that here several massulæ are actually seen anchored to the fibrils, which are exceedingly well preserved (Pl. XXVIII, Fig. 4). It is interesting to know that in this feature of the life-history our early Tertiary form has foreshadowed the condition in the modern species as described by Strasburger (1873, pl. 6, fig. 98) and Campbell (1893, pl. 9, fig. 75) for *A. filiculoides*, by Berggren (fig. 2, pl. 1) for *A. caroliniana*, and by Rao (1935, 190, Figs. 64, 72) for *A. pinnata*.

Fig. 1 gives a general idea of the specimen. Although in some details, especially the form of the group of floats at the top of the megasporocarp, it seems to differ slightly from the specimen previously figured, where the float-mass was more rounded at the top, there can be no doubt that it belongs to the same species. The difference is due partly to the mode of preservation, but chiefly to the fact that the opaque slice previously figured (Sahni and Rao, fig. 30; the same specimen was figured in

Sahni, 1940, pl. 1, fig. 9) was cut tangentially while the present specimen has been cut very near the median plane. The specimen now being described is a thin section *mounted between two coverslips* so that it can be examined under the high power of the microscope from both faces.

The body of the megasporocarp is now seen to be pear-shaped, with a pointed apex. It is about 500 μ long and 350 μ in maximum width. The persistent conical cap of the indusium is preserved in its place, as seen in the living species at the same stage of development. The free posterior edge of the cap is seen passing across the float-mass as a dark slightly curved line on both faces of the preparation. It is also seen projecting backwards on the right and left edges of the section (Figs. 1–3).

It is not possible to find the remains of the lower part of the indusium, which must have once covered the megasporangium: at this stage the part of the indusium over the region of the megaspore must have rotted away. Nor are any traces of the megasporangium wall visible: the spore itself is already exposed, with its weft of fibrils forming a felt about 18–20 μ thick over the spore wall. In the living forms these fibrils are known to be produced from the episporial layer of the megaspore wall (Strasburger, 1873; Rao, 1935, 184). In the fossil, too, I have been able to demonstrate their connection with the spore wall, although in the reproduction of Fig. 3 it has not been possible to show up this connection at all satisfactorily. There can be no doubt whatever as to the nature of these filaments : several massulæ are seen anchored to them by their glochidia in an unmistakable manner. One of these massulæ is seen lying at some little distance from the megaspore, its glochidia having pulled some of the filaments loose out of the otherwise compact felt (Figs. 1, 4).

The form of the anchor-like tip of the glochidium is identical with that described previously: the curved end-piece of the anchor is about 4 μ long between its two points, while the "shaft" is slightly constricted in the terminal part, beyond a characteristic enlargement at a point about 1 μ back from the end. Otherwise the shaft is uniformly thick, and it contains no septa. This form of the glochidium is distinct from those seen in the other known species. Strasburger has figured the glochidia in *A. filiculoides* (1873, fig. 85 *a*), *A. filiculoides* var. *rubra* (fig. 86 *a*), *A. caroliniana* (fig. 87), *A. pinnata* (fig. 88; see also Rao, 1935, figs. 55, 56) and *A. nilotica* (fig. 89). In the last two species the glochidium nature of these processes has been disputed by some botanists (*e.g.*, Strasburger, 59) but their development shows that their homology with the glochidia of the other species can hardly

be questioned. Moreover, at least in *A. pinnata*, they are known to function also in the same way, that is, as anchors for the massulæ (Rao, 190, pl. 20, figs. 64, 72).

It is evident that the glochidium can serve as an excellent diagnostic character, sufficient by itself to distinguish the different species. This fact is of special value to the palæobotanist who has usually to deal with small fragments.

The structure of the massulæ is identical with that already described. In the microspores I have not been able to find any definite evidence of germination, though it is at this stage, while the massulæ are floating about anchored to the megaspore, that the microspores might be expected to show signs of germination. In a free-lying massula (at × in fig. 2) two well-preserved microspores show a clear triradiate mark (see Fig. 6). The relatively thin spore wall is sometimes invaginated on one side.

Reverting to the megaspore, the wall is *ca.* 20 μ thick, apart from its covering felt of fibrils which in places is equally thick or even thicker (Fig. 4). The external surface of the megaspore wall, from which the fibrils spring, is rough, but the nature of the sculpturing is not possible to describe because the surface was ground off in preparing the section. The apex of the spore is produced like a broad pyramid about 50 μ in height which projects upwards between the floats, and corresponds to the triradiate mark or trilete of all tetrahedral spores. At the base of this pyramid the spore wall is expanded outwards in the form of a reflexed flange (Fig 2). The wall in this apical part of the spore is thinner, but the felt of fibrils is present also over this part, although it is covered by the floats. The length of the spore is *ca.* 297 μ, the maximum width *ca.* 272 μ, including the thickness of the felt of fibrils.

The megaspore is seen dehisced at the apex, but the opening is still very narrow, and there is no trace of the female prothallus.* At first sight it appears as if the spore cavity is divided by a very definite curved wall into two parts, an upper and a lower. And this might suggest a comparison with the condition in the modern species of *Azolla*, in fact in all heterosporous ferns in which, as is well known, the female prothallus is developed only in the upper part of the spore cavity, the lower part being left empty. But a careful examination shows that this interpretation is not correct. In

* Since this paper was sent to the press several other megaspores have been discovered with the massulæ anchored to the fibrils. In one of them the dehisced megaspore, seen from the top, shows a gaping trilete but unfortunately the female prothallus is not preserved.

the first place, in the modern species of *Azolla* and in the other heterosporous ferns the female prothallus is never cut off by such a well-defined wall from the lower part of the spore cavity (see the works of Rao, 1935, 187; Berggren, figs. 13, 16, 21; and Campbell, 1893, figs. 43–49). Moreover, the way in which this wall is seen to curve round at the two sides so as to meet the thick outer wall of the megaspore shows that it must be the elastic inner wall of the spore, accidentally caved in by pressure from outside, like a thin rubber ball poked by a finger. On examining the section from the other face (Fig. 3) it is seen that this membrane is in reality much thinner than would appear from its obliquely cut view in Fig. 1. The section is too thick to show clearly the line of separation between the two walls, but there can be no doubt about the explanation here given.

All these facts suggest that the specimen before us represents a very early stage in the germination of the megaspore—a stage soon after the spore dehisced but long before the fully developed female prothallus was formed. This would also explain why none of the microspores in the attached massulæ show any signs of germination: any antherozoids liberated at this stage would be far too premature.

The thickness of the inner wall as seen in the photograph is much exaggerated by the fact that the section itself is rather thick and the wall is not cut normally. But allowing for all this, and by focussing the actual cut edge, I conclude that the real thickness must be about half of the apparent thickness seen in Fig. 1.

The floats are exceedingly well preserved, but their exact number is difficult to ascertain in a longitudinal section. At a first examination there appear to have been at least four of them, because at least four distinct outlines of floats are seen. Two are visible on the right-hand side of the sporocarp, at two different depths of focus; and two on the left side, again at different levels (Fig. 1). It is possible, however, that these four outlines belong to only two floats, one on either side. The two outlines on each side may only represent the cut faces of one and the same float, separated by the thickness of the section, which is considerable.

In addition to these floats (two or four as the case may be) the contours of two more lobes are visible on the other face of the section (Fig. 3); but here, again, only small portions of the floats are visible and it is not certain that one or both of them are not, in fact, parts of those seen at the right and left sides of the sporocarp. It is probable that in preparing the thin slice portions of one or more of the floats have been ground off, but it seems unlikely that the whole of any one float has been lost.

Thus, while we cannot tell the exact number with certainty, at least it seems clear that the number cannot have been a large one: there may have been four, but more probably there were only three, which is the number characteristic of those living species in which anchor-tipped glochidia are developed.

The floats illustrate remarkably well their essential similarity in structure to the massulæ. Not only is the sponge-like pseudo-cellular mass of the float clearly preserved, but even the few dark knot-like bodies scattered through the mass of the float are prominently seen (Figs. 1, 3), exactly as they have been figured in *A. filiculoides* and other modern forms by Strasburger (pl. 6, figs. 94, 95), by Campbell (1893, pl. 8, fig. 35) and by others. As described previously, the group of floats comes down rather low, like a deep helmet, over the megaspore. Between the floats the axial canal, into the base of which the pyramid of the megaspore projects, is seen extending apexwards, but I have not been able to make out the narrow opening of the indusium to the exterior. The section, however, is not quite a median one; this probably explains why the thickening of the indusium tip with its specially large cells, which is characteristic of all living species, is not seen.

In view of the extraordinarily fine state of preservation hopes were raised of finding evidence of a symbiotic *Anabæna*, but even the most careful search in the region below the tip of the indusium has been futile.

Discussion

The first and most striking fact concerning this specimen is the great perfection in which it is preserved. This helps us to see that in this early Tertiary *Azolla*, which is definitely the oldest known species in the genus, some of the most intimate details of the structure, and the way in which the massulæ became anchored to the megaspore, are identical with those found in modern species. Considering that this plant lived about 60–70 million years ago it affords an impressive example of the persistence through the ages of a highly specialised type of behaviour during the reproductive phase of the life-history of a genus. Nor have we any special reason to doubt that even in Eocene times the different phases of the life cycle of this genus in the Deccan responded to the phases of the seasonal cycle in the same way as they do to-day in the modern Deccan species, *A. pinnata*. Therefore the fact that the massulæ of the modern species become anchored to the megaspore towards the month of September (Rao, 1935), warrants the suggestion that the specimen here described lived in a lake which was probably overwhelmed by a volcanic outburst during the autumn. Would that some geologist came forward to either confirm or refute the time of occurrence

of a volcanic eruption at Sausar as indicated by this little speck of vegetable matter !

The modern species of *Azolla* are generally grouped into two sections (Baker, 1887, 137):

(1) Section *Euazolla*, in which the megasporocarp has only three floats and the massulæ have well-developed anchor-tipped glochidia attached on all sides of them. This section includes *A. filiculoides*, *A. rubra* (very similar to *A. filiculoides* and by Strasburger regarded as a variety) and *A. caroliniana*. Of these *A. filiculoides* is also known in the fossil condition from the Pleistocene of Holland (van Baren, 1919; Florschütz, 1928).

(2) Section *Rhizosperma*, in which the floats are more numerous, and the poorly developed glochidia devoid of anchor-tips and placed only on one side of the massulæ. Here belong *A. pinnata* and *A. nilotica*. Of these forms *A. pinnata* has been recorded from the Pleistocene of Holland (Florschütz, 1928).

Of the extinct species, the affinities of *A. intertrappea* are definitely on the side of *Euazolla*, because of the presence of anchor-tipped glochidia and the small number of floats. The slightly younger species *A. prisca*, described by Reid and Chandler (1926) from the Oligocene of England, also resembles *Euazolla* in having well-developed glochidia with anchor-tips, but it recalls *Rhizosperma* in possessing nine floats. This extinct species therefore to some extent obliterates the two-fold classification of the genus. A third extinct species, recorded by Florschütz (1935) from Interglacial beds in Holland, resembles the section *Rhizosperma* in having nine floats on the megaspore. This Pleistocene species has been named *A. tegeliensis* but so far as I know it has not yet been described or figured. Florschütz (1935) also mentions briefly a fourth species, *A.* cf. *pinnata*, said to have been recorded by Nikitin (1928) from the Middle Pliocene of Central Russia and apparently since regarded by Nikitin as a form distinct from *A. pinnata*. I have not seen any description or figures. Our knowledge of *A. tertiaria* (Berry, 1927, 4) is too imperfect to justify any speculation as to its affinities.

The Deccan species is thus geologically the oldest and at the same time one of the most completely known of the fossil forms of *Azolla*.

My thanks are due to Mr. R. V. Sitholey for the photographs illustrating this paper.

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* These papers have not been seen by me.

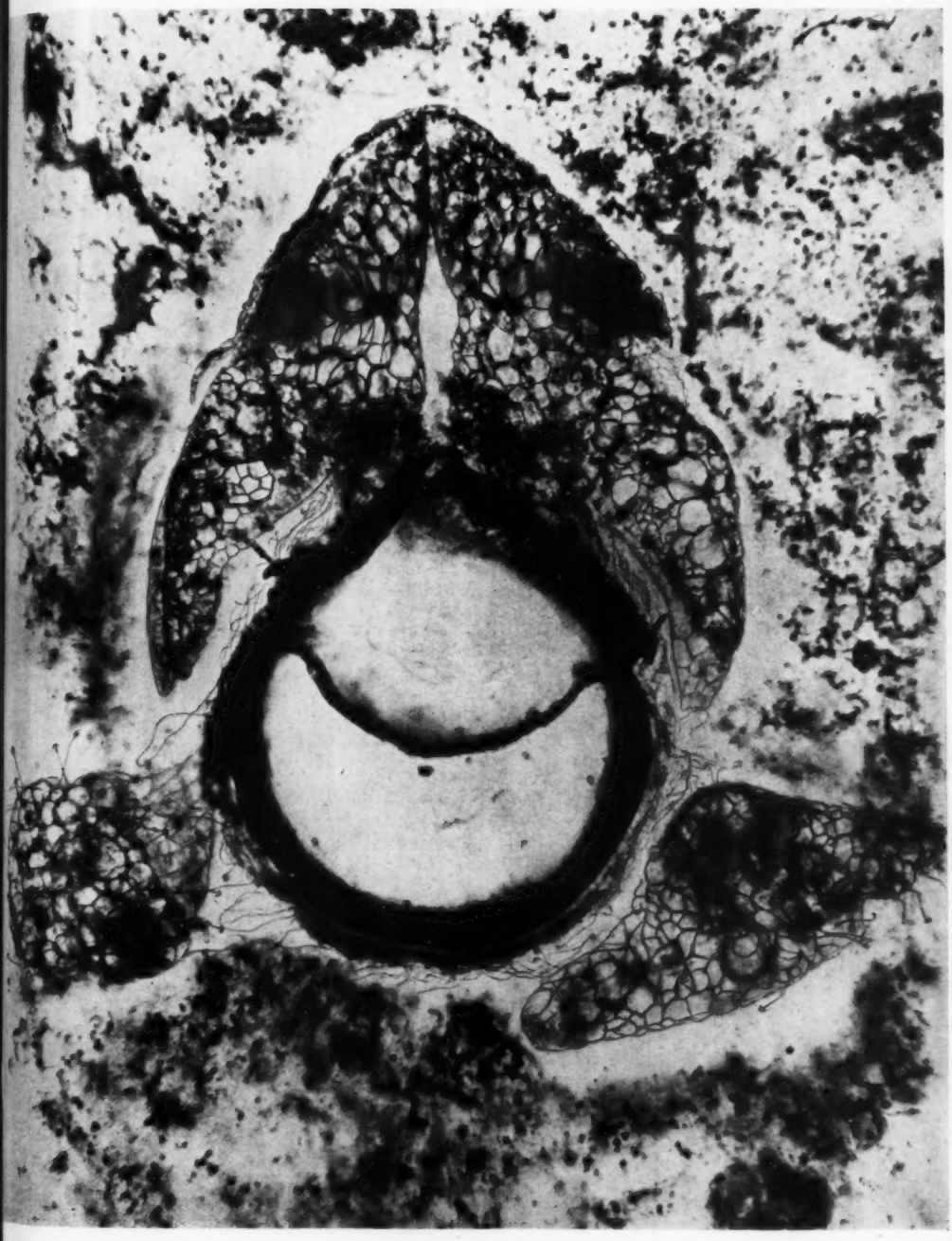

FIG. 1. × 292

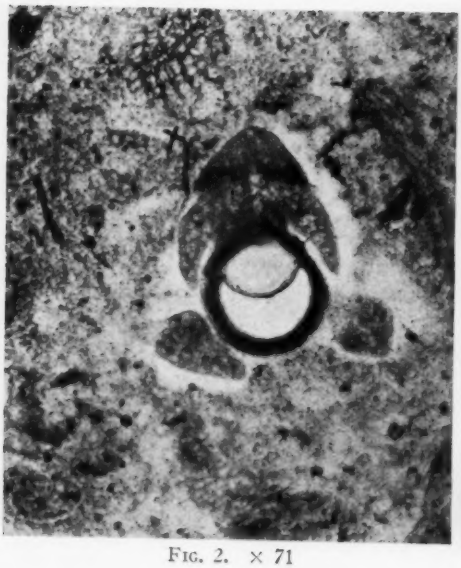

FIG. 2. × 71

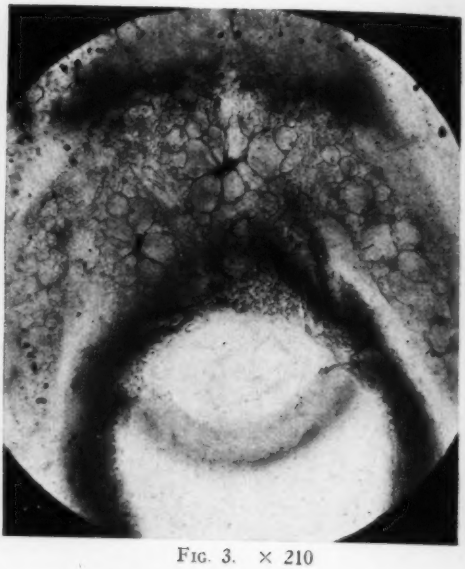

FIG. 3. × 210

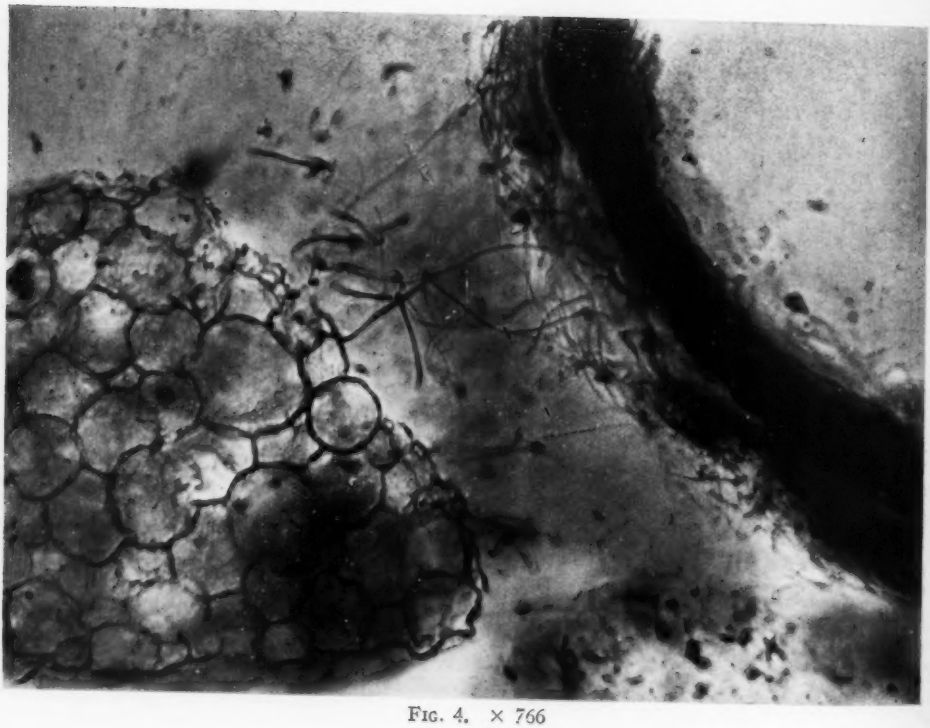

FIG. 4. × 766

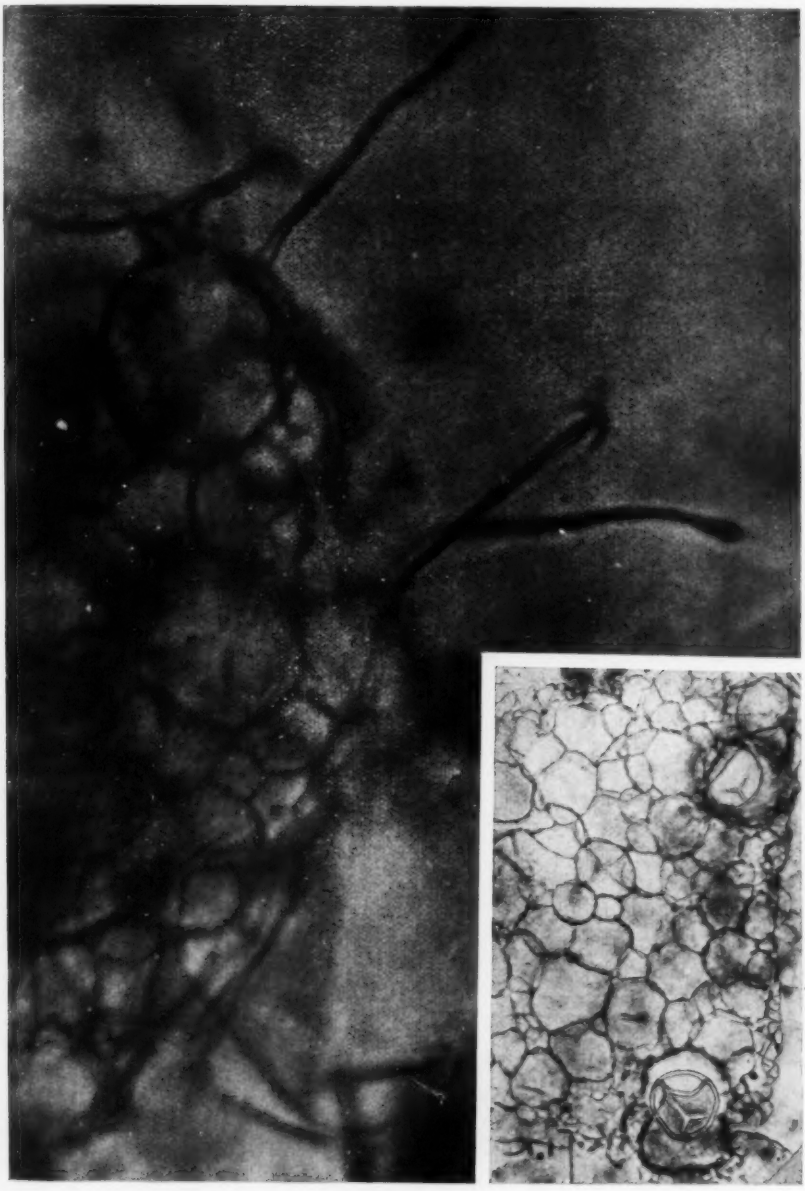

FIG. 5. × 1875

FIG. 6. × ca. 450

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EXPLANATION OF PLATES

Except Figs. 1 and 6 all the figures are from untouched photographs.

PLATE XXVI

FIG. 1. General view of specimen showing massulæ anchored to fibrils of megaspore. Fibrils are present also between the floats and the megaspore wall, and (right) on the outer side of a float. Details partially intensified in pencil on a matt photoprint. × 292.

PLATE XXVII

FIG. 2. General view of section from the other face, to show the associated plant fragments. To the left of × is a free massula, enlarged further in Fig. 6. × 71.

FIG. 3. Upper part of sporocarp, from the same face as in Fig. 2, to show details of two floats, each with a black "knot" in the centre. In the space between the floats some fibrils are seen connected to the megaspore wall, but in the reproduction of the photograph the connexion has been obliterated. The inner spore wall (invaginated into the spore cavity) is seen in oblique surface view. × 210.

FIG. 4. Details of lower left-hand part of megasporocarp, to show thick felt of fibrils over the spore wall, and glochidia entangled with fibrils. × 766.

PLATE XXVIII

FIG. 5. Part of a massula from Fig. 1 (bottom left) to show typical form of glochidium-tip and attachment of glochidia to the pseudo-cellular mass. × 1875.

FIG. 6. Part of a free-lying massula showing two microspores with triradiate marks. A third spore, without the mark, is seen towards the top left. Print partially re-touched to intensify the details. × *ca.* 450.

OBSERVATIONS ON THE DEVELOPMENT OF *ARIUS JELLA* (DAY)

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Received October 14, 1941

(Communicated by Prof. R. Gopala Aiyar)

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1. Introduction

It is well known that the richly yolked Teleostean eggs show a complete segregation of protoplasm and yolk and that the segmentation is typically meroblastic in character. Based on the above observation it has been suggested that in the early Teleosts the eggs should have been large in size richly provided with yolk. *Arius jella* that migrates from backwater to backwater through the sea, has eggs possessing an immense quantity of yolk and exhibits the phenomenon of parental care. It was thought that a knowledge of its developmental history would be interesting. Below are recorded some observations on the development of this form till the absorption of the yolk sac and the release of the well-developed young ones from the oral cavity of the male parent.

The eggs of the genus *Arius* are fairly large and they vary from 3 mm. in diameter in *Arius australis* (Semon, 1893) to 10 mm. in *Arius bœkii* and to 18 mm. in *Arius commersonii* (Kerr, 1919). The eggs of *Arius jella* measure 10·5 mm. on an average, the smallest egg measuring 9·5 mm. and the largest 11·5 mm. in diameter. The mature oocytes of *Osteogeniosus militaris*, a fish belonging to the family Siluridæ, are large, measuring 9 mm. in diameter and resemble those of *Arius jella*. The interesting phenomenon of oral gestation is also noticed in some of the South American Catfishes as *Galichthys felis* (Lee, 1935) and in *Hexanematichthys* (Gill, 1905). Males of the genus *Osteogeniosus* have also been observed to carry 15 to 20 eggs in their mouth (Day, 1889).

2. *Parental Care*

The phenomenon of parental care exhibited by this fish is exceedingly interesting. As soon as the eggs are laid by the female, the male fertilises and transfers them to his mouth. The oral gestation lasts for a very long time, the embryos remaining in the mouth till the yolk sac is not visible. During this period, the male parent actually starves. The alimentary canal becomes very thin and wiry with absolutely no food content. The number of eggs carried by a single male varies from 30 to 40 on an average. Probably due to fright these fishes blow out the embryos when caught. Therefore in order to get a specimen with embryos in the pharyngeal cavity, the procedure adopted was to hold the pectoral fins close to the body, thus preventing the fish from opening its mouth.

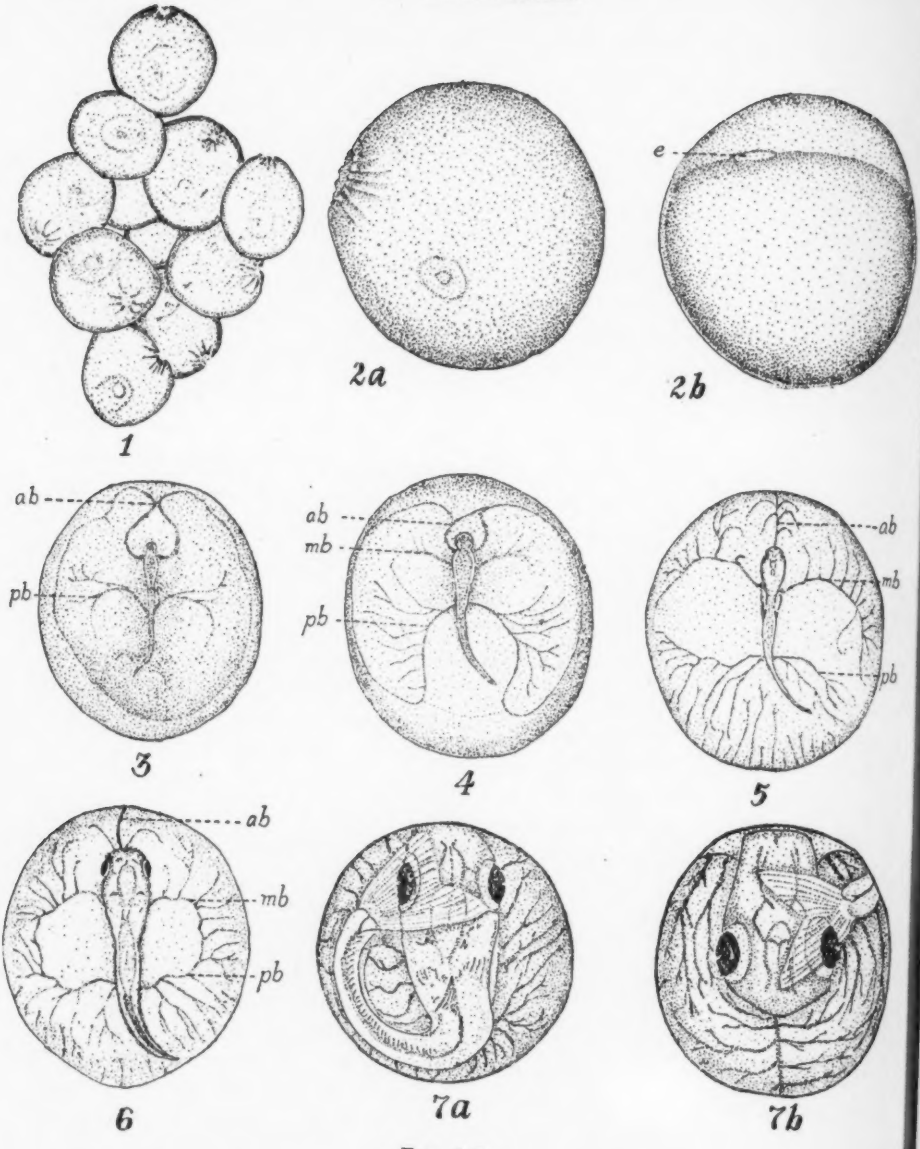
3. *Early Development till Hatching*

The embryos as soon as they were collected from the mouth of the male parent were placed in broad shallow dishes with brackish water just enough to cover them. The water was changed daily. In this condition, the eggs remained healthy. The embryos that hatched out in the laboratory thrived well only when kept just submerged in a little quantity of water; they occasionally rest on the substratum protruding their heads outside the water.

The breeding season is fairly extensive and eggs have been obtained in fair numbers from August to March; the ripe males and females are fairly large in size measuring 12″ in length or more. Judging from the very early embryos collected in the evenings, it is probable that the egg-laying takes place at that time. The eggs are laid in a bunch held together by mucus. When the eggs are extruded by the female, they are heavily yolked, rounded spheres, light yellowish green in colour, resembling grapes and measuring 10 to 10·5 mm. in diameter (Figs. 1 and 2). They are held together in a bunch for some time and they get separated after a few hours probably due to the action of water. The ripe ovary of a female measuring 14″ contained 50 well-developed ova and numerous immature ones measuring 1·5 to 2 mm. in diameter which were quite transparent. The membrane surrounding the mature egg is very thick and is about 1 mm. in thickness.

Embryo 60 hours old (Fig. 3): The embryo is 5 mm. long with the head in the form of a rounded swelling curved down in front. Surrounding the head and in the region following it, there are two circular transparent areas. Even at this stage, the embryo is seen moving its tail occasionally.

Embryo 80 hours old (Fig. 4): Length 7 to 7·5 mm. Pigment is formed in the eyes which appear as two black specks. The vascular area covers two-



FIGS. 1–7

FIG. 1. An egg bunch showing a few eggs soon after they were laid. FIGS. 2*a* and 2*b*. Egg that has separated from the bunch a few hours after it is laid. FIG. 3. Embryo that is 60 hours old. FIG. 4. Embryo 80 hours old. FIG. 5. Embryo 5 days old. FIG. 6. Embryo 15 days old. FIG. 7. Embryo 30 days old, just before hatching. (*a*) Dorsal view. (*b*) Anterior view.

thirds of the yolk mass and blood vessels with red blood corpuscles have already made their appearance. A pair of blood vessels taking origin just posterior and ventral to the head and running close together anteriorly for a little distance in front, curve outwards along the margin of the blastoderm. This is the anterior pair of blood vessels.

Embryo 90 hours old: Length 8 mm. The three main divisions of the brain become clear at this stage. Of the two blood vessels of the anterior pair the right one is less developed and gets absorbed early whereas the left one after running a little distance anteriorly divides into right and left branches that traverse the edge of the blastoderm. Another pair of blood vessels make their appearance in the posterior region. This is the posterior pair of blood vessels. The branches of these are distributed in the hinder portion of the yolk sac. The heart beat is clearly seen. The pectoral fins appear as small buds at the sides just behind the head region.

Embryo 5 days old (Fig. 5): Length 10 mm. The colour of the yolk is orange yellow. The vascular area has completely covered the yolk mass. The eyes are well marked black spots. A third pair of blood vessels joining the heart at its sides is formed in between the two pairs of blood vessels already formed and they are almost connected with the posterior pair of blood vessels at the sides. This is the middle pair of bloodvessels. Blood vessels are also formed in the head of the embryo and there is a median blood vessel running posteriorly right up to the tail. Just where the middle pair of blood vessels come out of the body of the developing young, a mass of vascular tissue becomes marked out. It is 2·5 to 2·8 mm. long; this is the beginning of the formation of the liver. The maxillary barbels arise as two prominences at the sides at the angle of the jaw in the maxillary region. The pectoral fin buds are clearly seen and the first dorsal fin is marked out as a thin outgrowth in the region following the head of the embryo.

Embryo 10 days old: Length 13 mm. The blood vessels that are distributed in the yolk mass are bright in colour and the stream of red blood corpuscles in these vessels could be clearly observed with a hand lens. The yolk is reddish yellow. At this stage the embryo shows movements of its head and tail. The two pairs of mandibular barbels have made their appearance. The ventral, the anal and the caudal fins have also been formed. The number of the heart beats per minute is 68.

Embryo 15 days old (Fig. 6): Length 15 mm. The middle pair of blood vessels is not so bright and clear as the better developed posterior pair. The three pairs of barbels show distinct growth in length.

Embryo 18 days old: Length 16·5 to 17 mm. At this stage, of the two blood vessels of the middle pair, the right one shows brighter colour and is

more pronounced than the left. Of the two of the posterior pair, the reverse is noticed: the left blood vessel is more pronounced than the right.

Embryo 20 days old: Length 18 mm. With the gradual absorption of the yolk sac, the anterior pair of blood vessels supplying the head region of the embryo disappear. The visceral arches and the blood vessels supplying them are well developed and the movements of these arches could be seen. All the fins are formed. The pectorals grow more rapidly than the others. The maxillary barbel at this stage reaches the posterior end of the head. The alimentary canal is coiled; the air bladder and the generative organs are clearly noticed.

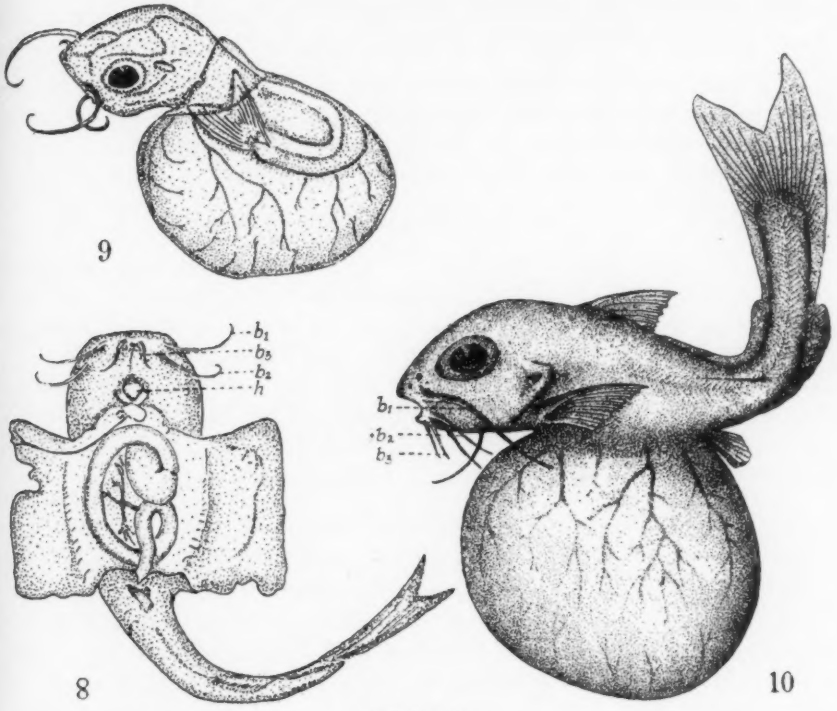
Embryo 25–30 days old (Figs. 7 and 8): Length 25 mm. The head is accommodated in a hollow cavity in the yolk sac the tail curving round and meeting the head. The embryo can shift the tail from the right to the left occasionally. The rolling of the eyes and the beating of the heart are prominent movements noticeable. The number of heart beats per minute is 60.

Hatching: Hatching takes place on the 30th or 31st day (Fig. 9). Prior to hatching, the head and the tail exhibit jerky movements. Due to these jerky movements the outer envelope splits exposing the head. The embryo continues to wriggle and in a short time the caudal fin also becomes free of the test. This is followed by a period of quiescence, during which the only appreciable movement noticed is the occasional rolling of the eyes. After about 10 minutes, the embryo straightens itself with a jerk and a twist, which while freeing it from the test sends it spinning along the bottom of the dish. This was noticed in those embryos that hatched out in the laboratory—outside the pharyngeal cavity of the parent. But inside the mouth of the male are seen hatched out embryos that are 15 to 20 days old, judging from their development.

4. Later Development

The newly hatched larva (Fig. 10): Length 25 to 26 mm. The head is 7 mm. long and 5 mm. broad. All the three pairs of barbels are well developed. The eyes are dark black spots, 2 mm. in diameter with an outer lid that has a golden shine about it. The yolk sac is a big rounded structure measuring 9 to 10 mm., extending from the region following the heart to the portion just anterior to the anus. The tail of the newly hatched young is curved upwards but it becomes straight after a few hours. The embryo is fleshy in colour with black eyes and grey barbels. Melanophores make their appearance on the following day in the dorsal portion of the head at the region following the gills and at the base of the spine of the first dorsal fin.

The chief changes that are noted in the later stages of the embryo are growth in size, changes in colour and the diminution in size of the yolk



FIGS. 8–10

FIG. 8. Embryo showing the internal viscera after it has been separated from the yolk sac and the test removed—30 days old. FIG. 9. Embryo 30 days old. Just hatching out. FIG. 10. Newly hatched embryo—one month old.

sac. Fifteen days after hatching (Fig. 11) the embryo shows rosy patches at the sides of the head region. The lower half of the body is dull white; the

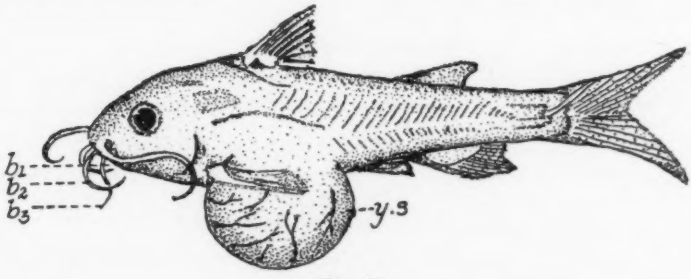


FIG. 11

Free swimming embryo—15 days after hatching, showing the yolk sac outside.

upper half shows a greenish hue. The mandibular barbels are streaked with white in their anterior face, whereas the other barbels are black.

The yolk sac is absorbed in the course of a month after hatching. As it gets absorbed, the posterior pair of blood vessels supplying the hinder region of the sac disappears. The anterior median blood vessel is slightly turned towards the right side. The prolonged period of absorption of the yolk sac is probably due to the large quantity of yolk.

5. *Summary*

The development of the egg of *Arius jella* is very slow and the time taken is two months. The embryo is marked out in the egg on the third day; the eyes make their appearance on the following day. The growth of the embryo is gradual till it is 20 days old; in the following ten days it is rapid and the young hatches out on the 30th or 31st day after the egg is laid. From the time of hatching it takes another month to develop as a young one resembling the adult fish.

6. *Acknowledgement*

It gives me very great pleasure to acknowledge my deep indebtedness to Prof. R. Gopala Aiyar, Director, University Zoological Research Laboratory, Madras, for his invaluable criticisms, constant help and kind encouragement. My thanks are also due to Dr. Subramaniam for his kind help. I am also grateful to the University of Madras for having allowed me to work in the Laboratory.

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KEY TO THE LETTERING

ab, Anterior pair of blood vessels; b_1, Maxillary barbel; b_2, First mandibular barbel; b_3, Second mandibular barbel; *e*, Embryonic area; *h*, heart; *mb*, Median pair of blood vessels; *pb*, Posterior pair of blood vessels; *y.s.*, Yolk sac.

CYTOLOGICAL STUDIES IN INDIAN PARASITIC PLANTS

I. The Cytology of *Striga*

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Received July 23, 1941

Very few of the Indian parasitic angiosperms have been investigated cytologically and even the chromosome numbers of most of them are not known. The present paper deals with the cytology of four species of *Striga*. Studies on other parasitic plants are in progress.

Material and Methods

Materials for the present study were collected locally. Three species, *S. lutea* Lour., *S. densiflora* Benth., and *S. euphrasioides* Benth., were collected from pot-cultures used for another investigation* and the host plant in all these cases was *Sorghum vulgare*. These three species of *Striga* are very similar to each other and bear a number of branches with numerous green leaves. They are hemi-parasites, while the fourth species, *S. orobanchoides* Benth., was collected locally from a marshy field. Preliminary examination in acetocarmine showed that the meiotic divisions take place when the buds are very small. The fourth bud above the flower which had opened in the morning of the day on which fixation was made, usually showed the meiotic divisions in at least one of the four anthers. Buds of this stage and slightly older as well as a little younger were taken and the sepals removed and after pretreatment with Carnoy's fluid for one minute were transferred to a chrome-acetic-formalin solution (2% chromic acid 5 c.c., 12% glacial acetic acid 5 c.c. and neutral formalin 5 c.c.) with a trace of saponin added. After the usual subsequent treatments, sections were cut at a thickness of 12 μ and stained in iodine gentian violet.

Observations

The somatic chromosome number was determined from metphase plates of ovary cells, in which the chromosomes were well spaced out. A certain degree of size variation in chromosomes was apparent even in

* Scheme of Research on the attack of *Striga* on Jowar.

adjoining metaphase plates in the same section. This appeared to be associated with size differences in the two cells, larger cells having chromosomes more evenly spaced out and more slender while smaller cells have chromosomes more crowded and more condensed. Chromosome counts at meiosis were made from polar views of I and II metaphases.

Mitosis :

S. densiflora.—Somatic chromosome number of this species is found to be $2n = 40$. Fig. 4 shows a metaphase plate in which the chromosomes are rather condensed. Two of the forty chromosomes are rather longer than the rest.

S. euphrasioides.—Fig. 5 shows a metaphase plate in which forty chromosomes are clearly counted. Here also two chromosomes are markedly longer than any of the other thirty-eight.

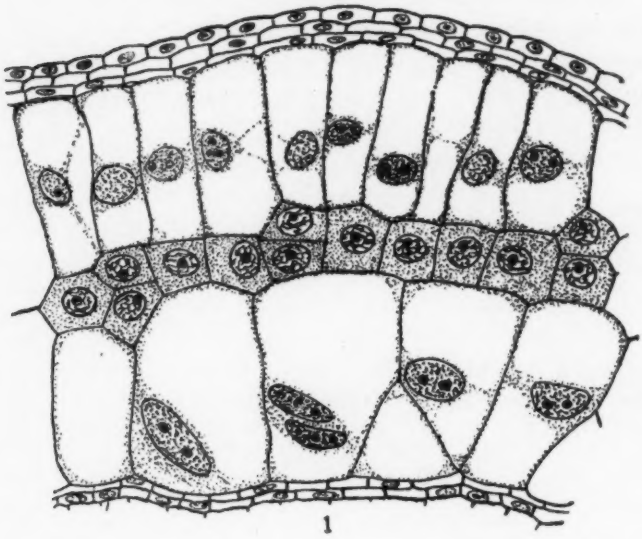
S. lutea.—Metaphase plates with chromosomes well spaced out were not observed, but the number as determined from a few plates in which chromosomes were rather crowded showed that the $2n$ number is forty in this species also.

S. orobanchoides.—While the chromosome number in this species also is $2n = 40$, the chromosomes appear to be larger than in the other three species (Fig. 6). Two long chromosomes are seen in this complement also.

Meiosis:

Preparations showing all the different stages of meiosis were obtained in the case of *S. densiflora* and *S. euphrasioides*, while in the other two species a few of the stages were not observed. Details of the meiotic division are mentioned only very briefly as no very unusual aspects were observed.

In all the four species all the four anthers are fertile. The anthers are two-lobed in cross section. The primary archesporial layer appears very early in the ontogeny of the anther, and by periclinal division it gives rise to the primary wall layer and the primary sporogenous layer. The former gives rise to three layers of cells, the two near the epidermis being narrow and elongated, while the third surrounding the sporogenous layer constitutes the tapetum. The tapetum is very conspicuous on both sides of the sporogenous layer, by its very large size and prominent nuclei. In Fig. 1, a few of the tapetal cells show two nuclei in each cell, while the spore mother cell nuclei are still in the early stages of meiosis. In cross sections and in vertical sections through certain planes, the sporogenous layer is found to be composed of single row of cells while at certain regions they are two cells thick. This shows that the primary sporogenous cells undergo



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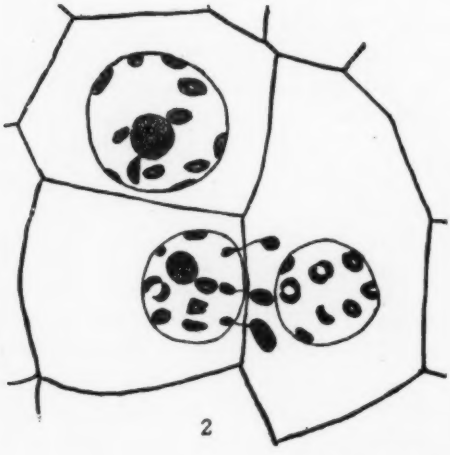

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3

TEXT-FIGS. 1—3

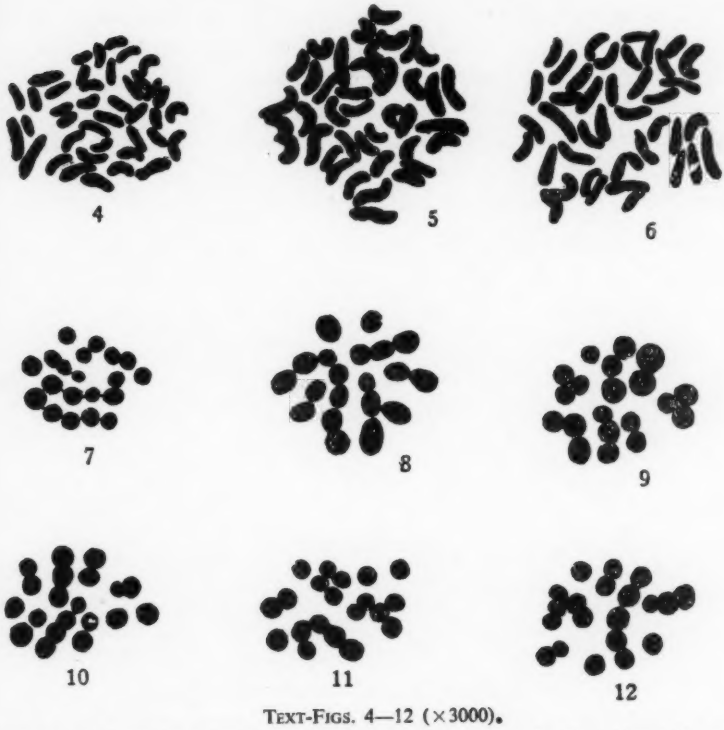
FIG. 1. *S. densiflora.*– Part of longitudinal section of an anther showing the large tapetal cells in some of which the nuclei have already divided. The pollen mother cell nuclei are in the early prophase of meiosis. ×540. FIG. 2. Chromatin extrusion in *S. euphrasioides*. ×2000. FIG. 3. *S. densiflora.*—A nucleus at diakinesis showing three bivalents in contact with nucleolus. One of the three bivalents is satellited and the satellites are lying on the nucleolus. ×3000.

mitosis only at certain regions, while at other regions they directly function as spore mother cells.

Prophase pairing is normal and at diplotene we find bivalents equidistant from one another. They condense further and at diakinesis the bivalents appear to be held together by two terminal chiasmata, giving rise to a ring-like appearance. In Fig. 3 showing a nucleus at diakinesis in *S. densiflora* three bivalents appear to be in contact with the nucleolus, but only one is satellited and intimately connected to it. Chromatin extrusion was observed in some pollen mother cells of one anther of *S. euphrasioides* (Fig. 2).

The equidistant orientation of bivalents in the periphery of the nucleus at diakinesis must be due either to a repulsion between bivalents as suggested by Lawrence (1931) or due to a repulsion force acting from the centre of the nucleus. However, this arrangement disappears with the loss of the nuclear membrane at pro-metaphase and the bivalents move towards the equator of the cell where they arrange into a plate. During this movement from the periphery to the centre some bivalents naturally come closer to each other. Whether these bivalents maintain this close association to give the appearance described as " secondary association " or whether there is a re-orientation of bivalents before metapahse plate is formed could not be determined. But the question arises as to how far secondary associations represent attraction due to residual homology and how far chance proximity of bivalents during pro-metaphase movement contributes to this appearance. It was found in some cases that, probably due to the action of fixatives or due to other undetermined causes, metaphase bivalents come together much more closely than is normal at that stage. This did not suggest a general collapse of the bivalents, as only a few bivalents are so closely associated while others are well spaced.

In *S. densiflora* and *S. euphrasioides* the haploid number is found to be twenty (Figs. 8–12). The haploid number in *S. lutea* was determined from a polar view of I anaphase and in *S. orobanchoides* from diakinesis nuclei. In both these species also the haploid number was found to be twenty. Figs. 8 and 9 show I metaphase plates from *S. euphrasioides* and Figs. 10–12 show the same stage from *S. densiflora*. In both cases very clear secondary association of the bivalents into eight groups is seen.



TEXT-FIGS. 4—12 (×3000).

FIG. 4. *S. densiflora*.—Somatic metaphase. $2n = 40$. FIG. 5. *S. euphrasioides*.—Somatic metaphase, $2n = 40$. FIG. 6. *S. orobanchoides*.—Somatic metaphase, $2n = 40$. FIG. 7. *S. lutea*.—Polar view of I anaphase showing 20 chromosomes. FIGS. 8 and 9. *S. euphrasioides*.—I metaphase showing 20 bivalents. Secondary association into 7 and 8 groups respectively. FIGS. 10, 11 and 12. *S. densiflora*.—I metaphase showing 20 bivalents in each. Fig. 10 shows secondary association into 10 groups, while in Figs. 11 and 12 the typical arrangement into 8 groups is shown.

TABLE I

Analysis of I metaphase secondary association in S. densiflora

Number of bivalent groups	Number of I metaphase plates	Frequency percentage
6	1	3·3
7	2	6·6
8	16	52·8
9	5	16·5
10	2	6·6
11	1	3·3
12	3	10·0
Total	30	

This was the most frequent type of grouping found in a count of thirty metaphase plates in *S. densiflora* and twelve metaphase plates in *S. euphrasioides*. A few cases were observed where the twenty bivalents formed into groups of 10, 11 or 12 (*cf.* Fig. 10), while very rarely groups of 6 and 7 were also noticed. The secondary association is maintained at anaphase I and metaphase II as well, as observed by Raghavan and Srinivasan (1940) in *Angelonia*, another genus of the Scrophulariaceæ.

Anaphasic separation is very regular and the chromosomes move as one plate so much so the metaphase association could be followed in some polar views of anaphase. The second division rapidly follows the formation of daughter nuclei in the first division. Cell wall formation takes place only after completion of the second nuclear division.

Discussion

Basic number of chromosomes in Striga.—The previous work on the cytology of the Scrophulariaceæ has been reviewed by Raghavan and Srinivasan (1940) who have also recorded the chromosome numbers in six genera and eight species belonging to this family. They have also discussed the significance of secondary association and the probable basic number of chromosomes in the family.

Secondary association of bivalents at I metaphase is now being used as a reliable criterion for determining secondary polyploidy in a genus and also to infer the basic chromosome number of the genus or family. Though it is generally accepted that secondary association is a manifestation of residual homology between the bivalents, it is not unlikely that chance position occupied by a bivalent during pro-metaphase movement may also lead to a closer orientation of two or more bivalents. This possibility together with the fact that the action of chemicals may also accentuate this appearance, necessitates great caution in the use of this evidence.

The meiotic prophase chromosomes are slender, long and coiled and still we normally find homologous chromosomes closely pairing. This shows that when there is an attraction of a sufficient magnitude between any two chromosomes, they could pair at the meiotic prophase in spite of possible mechanical difficulties due to presence of intervening chromosome strands or the very elongated and coiled condition of these chromosomes. Such being the case with respect to primary association, it is difficult to understand why, if secondary association is really due to a residual attraction (which must also be assumed to be of a specific nature if we are to draw any inference regarding ancestral homology from it) all bivalents capable of

secondary pairing do not do so frequently. Even if this secondary attraction is not of such a magnitude as that determining the prophase pairing, it should be expected, at least in cases where the chromosomes are few and small in size and the pollen mother cell of sufficient diameter—thus eliminating any mechanical barrier to bivalents coming together—that all bivalents capable of secondarily associating would do so. If this view is admissible, then the most frequent type of secondary association observed should be taken as representing the real maximum association to be expected on the basis of secondary homology, while those deviating from this may only be the result of other causes which apparently increase or decrease the number of bivalent groups. In this connection it is interesting to note the observations of Catcheside (1937) on *Brassica.* From counts of a very large number of I metaphase plates, he observed 80 cases of $3_{(2)} + 3_{(1)}$ association and 122 cases of $2_{(2)} + 5_{(1)}$ association. Though the latter showed the maximum frequency he has taken the former as indicating the basic number ($3+3=6$). But unlike some recent authors who have relied solely on the maximum association irrespective of the observed frequency, Catcheside considers higher association showing comparatively very low frequency as merely aberrant types of associations.

The most frequent type of secondary association found in a random selection of thirty metaphase plates in *S. densiflora* is into eight groups. A smaller sample of twelve metaphase plates examined in *S. euphrasioides* also showed nearly the same frequency percentage of association of bivalents into eight groups as in *S. densiflora.* Based on this finding and taking into account the fact that a large number of genera of the Scrophulariaceæ have eight as their basic chromosome number (Srinath, 1940), we are inclined to take eight as the basic number in *Striga* also, though it is strictly not the maximum association observed. On the basis of maximum association the number should be six; but this need be considered as an aberrant association only in view of the fact that it is unrelated to basic numbers in other allied genera and is seen only once in thirty plates examined. Raghavan and Srinivasan (1940) also found the most frequent type of secondary association in *Angelonia grandiflora* to be eight, though they found in two metaphase plates out of a total of thirty-one examined, a maximum association into five groups and from this took five as the basic number of the genus and even went so far as to suggest that this may be the primary basic number in the family. But a large number of genera of the Scrophulariaceæ have eight as their basic number and the present study shows yet another genus with the same basic number. If the most frequent association and not the maximum association is taken into account, then *Angelonia* too should be considered as having eight as the basic number.

For verifying the applicability of our suggestion, based mainly on theoretical considerations, that in plants showing secondary associations the most frequent type of such associations should be taken as indicating the basic number, it would be necessary to statistically analyse the chromosome arrangement in a number of closely related genera exhibiting secondary association and which in possessing only a small number of small-sized chromosomes are otherwise also favourable materials for such studies. An investigation on these lines is in progress in our laboratory.

Summary

The cytology of four species of *Striga*, *viz.*, *S. densiflora*, *S. euphrasioides*, *S. lutea* and *S. orobanchoides* is described. The chromosome numbers have been found to be $n = 20$ and $2n = 40$ in all the four species.

The basic number of chromosomes of the genus is shown to be eight. Based on theoretical considerations it is suggested that in using evidence from secondary associations the most frequent type of association should be taken as indicating the basic number.

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PRELIMINARY NOTES ON THE PARASITES OF THE SPOTTED AND THE PINK BOLLWORMS OF COTTON IN COIMBATORE*

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(Communicated by Rao Bahadur V. Ramanatha Ayyar)

From early times spotted bollworm parasites have received considerable attention in the Punjab. Investigations mostly centred round the then wrongly designated *Rhogas lefroyi* D. & G. No connected and comprehensive account of its biology appeared till a long time after. Meanwhile, C. T. Brues rectified the mistake and gave it the correct designation as *Microbracon lefroyi* D. & G. by which name it is now known. Fletcher (1919) and Ballard (1921) make only passing references about this insect. Husain and Mathur (1921) were the first to furnish an account of the ovipositional habits of the *Microbracon*. The same authors (1923) published a cogent account of the bollworm parasites of the Punjab subsequently. The contribution by Ramakrishna Ayyar (1926) was helpful in suggesting the occurrence of more than one species of *Microbracon* as being involved in the parasitization of the bollworms. More recently, Deshpande (1936) has given a short account of the several parasites of the spotted bollworms of South Gujarat. Ahmad and Ghulam (1939) have worked out the development of various stages of the parasite under varying conditions of temperature and humidity.

Much of what has been done and published mostly relates to the parasites of other provinces. Our knowledge of the South Indian bollworm parasites is still incomplete. The aim of the authors in bringing out this paper has been chiefly to add to the knowledge of these insects. The paper deals with the forms found in Coimbatore only. Intensive study and wider survey of the parasites are essential if a complete record is desired. Information on the parasites of other bollworms like *Rabila frontalis*, *Chloridea obsoleta*, has been omitted as they have seldom passed beyond the stages of minor pests of cotton.

* Paper presented at the Second Conference of Scientific Research Workers on Cotton in India, 1941.

A. Spotted Bollworm Parasites

The spotted bollworms *Earias fabia* and *E. insulana* that infest the cotton buds and bolls are subject to field parasitization by the following five parasites—*Microbracon lefroyi* D. & G., *Rhogas aligharensi* Quadri, *Bassus* n. sp., *Elasmus johnstoni* and *Actia hyalinata*. The following is a brief account of each:—

1. *Microbracon lefroyi*.—It appears early in the season from November onwards when the cotton plant just then starts budding and bolling, remains active till February and then fades out. It is not noticed in April and May or thereafter. The incidence of this species has never been marked and is about 2–3% (Table I). However, its incidence is comparatively higher in the shed buds than in the bolls. The total absence of the parasite late in the season may be due to dearth of buds and tender bolls which alone seem to be adapted for the insect to parasitize the host larvæ when inside. Even

TABLE I

Incidence of Microbracon lefroyi *D. & G. in the* Earias fabia *caterpillars in the shed buds and bolls of cotton (Cambodia)*

Year and Month	Buds and Flowers				Bolls			
	Total picked	Host population	No. parasitized	Percentage of parasitization	Total picked	Total population	No. parasitized	Percentage of parasitization
1938								
November II ..	679	3	1	33·3	313	23	..	..
December I ..	4,203	635	6	0·94	6,597	715	1	0·14
Do. II ..	2,373	55	2	3·63	18,483	307	2	0·64
1939								
January I ..	2,154	6	..	..	11,993	54	..	..
Do. II ..	114	1	..	..	2,386	16	..	..
May I ..	1,507	59	..	..	710	37	..	..
Do. II ..	7,917	125	..	..	1,597	142	..	..
June I ..	2,972	182	..	..	259	..	..	..
December II ..	1,563	99	..	..	1,037	113	3	2·65
1940								
January I ..	7,028	722	2	0·28	4,963	544	..	..
Do. II ..	5,910	57	..	..	3,687	59	1	1·60
February I ..	752	23	..	..	794	14	..	..
July II ..	560	19	..	..	407	1	..	..
August I ..	228	3	..	..	109	..	..	..

Note.—I and II refer to the first and second fortnights respectively.

at a time when the host population is very high in late December and early January it never shows up prominently and for this reason could not be regarded as efficient.

From the observations made over a period of two years it was found that this species would never accept an open or free larva for oviposition unless it is given in a closed cell. Our finding is in agreement with Deshpande (1936) but is in conflict with that of Husain and Mathur (1921) who state that the caterpillars given in small glass cubes are readily oviposited upon. Host larvæ placed in the improvised cells of cotton bolls were invariably parasitized; the cells are made by partially splitting open the bolls and scooping out the contents therein. The split halves can be held in position by a loop of thread after the host larva is placed in it. When such host larvæ are presented to the parasite it administers 5–6 stings to the host with its ovipositor from outside. The host as a result of these thrusts becomes immobile. The insect soon after tries to lay eggs. They are extruded through the ovipositor into the cell cavity and are often placed in groups close to the body of the host. It has no marked predeliction to lay eggs in any particular spot or position. Eggs have been seen from the head end to the tail end of the host in all positions. As a rule, 5–10 eggs are laid at a time. Each parasite is capable of laying a maximum of 75 eggs. The eggs are smooth and have a pearly sheen when freshly laid and are awl-shaped. Each egg is about 2 mm. long and nearly four times as long as broad with one end tapering.

Eggs hatch in 18–24 hours and hatchings emerge. They immediately crawl over the body of the host and fix themselves in any position and start feeding by imbibing the body juice of the host. In about three days they become full grown when they develop a light chocolate colour. Each grub spins a white silken cocoon close to the host and pupates therein. It emerges as an adult a week after pupation by biting holes in the cocoons.

Under Coimbatore conditions, the parasite takes 10–12 days to get through one generation.

The range of larval hosts for this insect is very limited in nature. It has been bred from *Adisura atkinsoni* in the pods of the housebeans, *Rabila frontalis* in the cotton bolls and *Earias* spp., in bhindi pods. It is rather remarkable that *Earias* spp. infesting the pods of *Hibiscus vitifolius*, *Abutilon hirtum* and *A. indicum* have never been parasitized by this species even though the alternate host plants co-existed with cotton in the vicinity, and were subject to similar environmental conditions. The bollworm infestation

in them was continuous and was fairly high. Under laboratory conditions, however, the parasite oviposited on other hosts like the pink bollworm and the *Abutilon gelechiad* with indifference; the development of the parasitic grubs was slow and in several instances, never passed beyond the cocooning stages. In a few cases, where the parasites were bred out, they bore a remarkable identity to the parent indicating that the parasite breeds true to type even on different host caterpillars.

The insect which is said to show considerable colour variations in Northern India is found here to conform to only one pattern of the B variety of Ramakrishna Ayyar (1926). No intermediate forms have so far been noted in the species either in the field or in the laboratory bred specimens. It occurs as a larval parasite of *Earias* spp. in cotton buds and flowers. It has not been reared so far from the pink bollworm though Ballard (1921) has stated so.

2. *Rhogas aligharensi*.—This is a specific parasite of the spotted bollworms that infest cotton buds, bolls and bhindi pods. The parasitic grubs are exclusively internal and do not come out of the body of the host. Like *M. lefroyi* it appears early in the season and continues to show up though sparingly till the end of April. Its period of maximum activity would appear to be in December and January when the host population is also at its highest. A single adult emerges from the affected host. Emergences of *Rhogas* and *Microbracon* are recorded in Table II. The proportion of both of them is about equal. On this basis there is reason to regard the former as more efficient, for, as many caterpillars are destroyed as there are parasites whereas, in the latter, the worms destroyed will be about a fourth of the total number of parasites that have issued. The affected caterpillars continue to feed till such time when they become sluggish and come out of the boll to become bloated. Nearly 7–8 days elapse before the adult comes out from the body of the host; it issues by biting a hole on the upper surface of the tail end.

3. *Bassus* n. sp.—The insect occurs as a specific parasite of *Earias* spp. caterpillars infesting tender and medium bolls of cotton; it has not been reared so far from the same hosts in other host plants. It is easily distinguishable by the black and red colouration of the body and by its very long ovipositor. The parasitic grub is an internal parasite and pupates within the body of the host. The adult issues after the latter has cocooned. As in *Rhogas* a single parasite results from each affected caterpillar. Table II gives an idea of its seasonal incidence. It appears in January and is not noted thereafter. It seems to be restricted in distribution in this Presidency and has not been noted to occur in other provinces.

TABLE II

Emergence record of parasites from shed cotton buds and bolls dumped in a cage on December 26 (1939) *and January* 14 *and* 25 (1940)

Date of Emergence		Nature of Parasites					
		Rhogas	*M. lefroyi*	*Apanteles*	*Bassus*	*Actia*	*Goniozus*
	1939						
December	28	1	..	..	..	..	..
	29	1	..	..	..	..	..
	30	1	..	..	..	..	..
	31	1	..	..	..	..	..
	1940						
January	1	1	..	..	..	..	..
	2	1	4	1	3	..	..
	3	1	3	3	..	..	..
	6	2	..	..	..	..	..
	7	2	3	3	1	1	..
	8	3	3	3	4	4	..
	9	6	2	4	..	5	..
	10	5	1	4	..	4	..
	12	11	2	3	..	3	..
	14	7	..	5	2	..	..
	16	2	..	2	3	..	..
	17	3	..	4	4	..	..
	18	3	3	10	..	..	..
	19	3	..	1	3	..	..
	20	2	4	2	2	..	..
	22	7	13	2	..	1	1
	23	6	16	5	..	1	..
	24	5	3	3	1	1	..
	25	7	13	1	..	1	..
	26	..	11	1	2	..	..
	27	3	4	..	..	..	..
	28	..	4	5	..	3	..
	29	7	5	1	2	3	..
	30	3	6	1	2	..	..
	31	..	..	1	..	..	..
	TOTAL	94	95	66	30	27	1

Note.—Microbracon greeni and *M. gelechidiphagus* were not recovered during the period

4. *Elasmus johnstoni.*—This black chalcid more commonly occurs as an ectolarval parasite of *Earias fabia* caterpillars infesting *H. vitifolius* pods. It is not much in evidence in the worms attacking cotton or bhindi. Its paucity in the latter must be attributable to its general habit of parasitizing the caterpillars that are surrounded by a thin wall of the cell. The

outer wall of the bolls and bhindi pods is tough and would therefore offer impediment to its effective parasitization. Under laboratory conditions, the insects show a marked preference to the cocooning caterpillars for parasitization; the silken cocoon offers the ideal condition for the parasite to reach the host with their ovipositors. They occur all through the year in limited numbers in *H. vitifolius* pods. Seven to ten is the normal number that is obtained from each host under the field conditions but in the laboratory, as many as 98 have been obtained from a single caterpillar. The population is predominantly female and their longevity does not exceed a fortnight when fed on sugar solution. Each parasite is capable of laying 8–18 eggs at a time. The host is paralysed by several thrusts with the ovipositor from outside and often the parasite takes a feed on the body fluid that exudes through the punctures caused by the stings. The eggs are laid in loose groups on any part of the body of the host; they are small and white. Pupation is naked within the host cocoon and the adults emerge within a week after pupation. Its life-history is completed within a period of 10–12 days. It is easy to breed this parasite in large numbers under laboratory conditions.

5. *Actia hyalinata.*—This small tachinid fly has been noted in very scanty numbers and has been bred out solely from the spotted bollworms. Not more than two were obtained from each affected host; the maggots come out of the body and transform into puparia from which the adults emerge after a week.

Spotted Bollworm Parasites Associated with Different Plant Hosts

The spotted bollworms that infest the pods of *H. vitifolius*, *A. indicum* and *A. hirtum* are attacked by *Microbracon greeni*, *Elasmus johnstoni* Ferr and *Polyodaspis compressiceps* Duda; in bhindi by *M. lefroyi*, *M. hebetor* (Say) and *Melcha nursei* Cam.

Of these, *M. greeni* is the most important. Its incidence varies from 8–43% in *H. vitifolius* pods (Table III). This weed maintains a uniform infestation of *Earias fabia* right through the year and the parasite is exclusively associated with the host caterpillars and flourishes in an equal degree all round the year. However, its parasitism in the larvæ infesting *A. hirtum* pods is considerably less even though the larval hosts continue to breed. The total absence of parasitism by *M. greeni* in the spotted bollworms in cotton and *vice versa*, namely, the absence of parasitism by *M. lefroyi* in that of *H. vitifolius*, *A. hirtum* and *A. indicum* coupled with disparity in size and coloration between the two species, would seem to suggest that really wo different species were involved, each being closely associated with its

TABLE III

Incidence of Microbracon greeni (*ash*) *in* Earias fabia *catterpillars in* Hibiscus vitifolius *pods for the years* 1937–40

Date of collection of pods		Number of pods collected	Host population	Number affected by parasites	Percentage of parasitization
1937					
November	3	58	19	3	15·79
	5	147	48	7	14·58
	9	229	40	4	10·00
	12	283	35	18	51·43
	19	140	12	..	..
	29	29	6	3	50·00
1938					
January	31	85	..	..	..
April	5	211	33	2	6·06
	22	106	6	..	..
June	21	88	8	2	25·00
	30	103	12	1	8·30
July	6	205	44	2	4·50
1939					
April	1	205	21	9	4·29
	8	24	2	2	100·00
	14	135	17	7	41·18
May	11	154	25	4	16·00
1940					
January	5	202	57	..	..
February	1	332	33	8	24·20
	7	253	29	2	6·90
July	27	606	81	1	1·23
August	10	572	65	12	18·46
September	4	375	75	11	14·61

Note.—Microbracon lefroyi has not been recovered.

own plant host complex. This assumption is further confirmed by their breeding true to type even when the hosts were altered. Lal (1939) is inclined to regard these two species as identical in the absence of any distinguishing morphological character; the color pattern was found to be a varying factor and could be altered at will by changing the temperatures at the time of development. One should agree with him if the species that had undergone transformation of color pattern could transmit the acquired character to its progeny without further recourse to the temperature. In all our rearings in the laboratory and under identical conditions, both the species retained their specific color pattern even where the hosts were different. One outstanding morphological character by which *M. greeni* of Coimbatore could

easily be marked off from the other is by its long ovipositor which is nearly one and a half times as long as the abdomen; the males exhibit a color pattern not noted to be present in the other species. The former has well-developed smoky clouds on all the abdominal segments. There was not much of variation in the duration of life-cycle in both the species.

Microbracon hebetor.—This is comparatively very small in size and was obtained only once from the caterpillars of *Earias fabia* infesting bhindi pods. Unlike *M. lefroyi* and *M. greeni* it would seem to freely accept the free forms of both the types of caterpillars for oviposition; it is most reluctant to oviposit on caterpillars when given in enclosed cells. Eighteen to twenty eggs are usually laid on the paralysed body of the host in any situation. They are lemon-yellow in colour. The grubs after full development spin cocoons near the host and pupate in them. It was also noted to be a parasite of the caterpillars of *Stomopteryx nerteria* in groundnut and *Antigastra catalaunalis* in gingelly.

Melcha nursei.—This ichneumonid was obtained only once as an internal parasite from *Earias fabia* caterpillars in the bhindi pods; it emerged after the host larva had cocooned.

Polyodaspis compressiceps.—This chloropid fly was obtained as a parasite on *Earias fabia* larvæ in *H. vitifolius* pods. Though its incidence is low it occurs all round the year. Not more than 3–4 maggots were obtained from a single host; they come out of the body and pupate within the pods close to the affected host.

B. Pink Bollworm Parasites

The caterpillars of *Platyedra gossypiella* Saund. have their own parasite complex. They are attacked in nature by the following parasites: *Goniozus* n. sp., *Apanteles pectinophoræ* and *Microbracon gelechidiphagus* Rank.

Goniozus n. sp.—This bethylid appears early in the season from November and does not show up prominently till late in May and June. It appears to be a blossom infesting type and it associated with the host infested cotton flowers. It appears to differ from the species recorded by Fletcher (1919); from the Plate given by him his bethylid appears to be a species of *Perisierola.* As high as 49% of the pink bollworms that infested the open and unopened flowers were found parasitized in July 1940. The insect seeks caterpillars of the fourth and fifth instar stages for egg-laying. It is predatory in habit and preys upon the blossom thrips and other young caterpillars found inside the flowers. It has been noted to feed on the nectar that collects in the nectar glands of the cotton flowers.

Though these bethylids attack the free caterpillars given to them in the laboratory and paralyse them prior to egg laying they suffer damage at

the hands of the hosts and become maimed but they seem to be quite well at home inside the flowers; cent. per cent. parasitization is recorded if the hosts are given with flowers. The female usually approaches the host from behind, seizes the host by the nape on the upper surface with its mandibles and curves round its abdomen. Series of stings are given in quick succession in the neighbourhood of the first thoracic segment on the ventral side. The worm soon quiets down and becomes motionless. The parasite escapes injury in the flowers as the filaments of the staminal column act as a bar to the host reaching the insect. Thereafter, the parasite takes pain to smoothen he intersegmental rings of the distal segments by running over its mandibles transversely across. The object for this behaviour would appear to be its desire to clear off any projecting setæ that might hinder the egg from being firmly glued to the segment.

Eggs are, as a rule, placed transversely across on the intersegmental rings of the abdominal segments 5–6, 6–7, 7–8, 8–9. It lays 6–13 eggs at a time; each segment carries 2–4 eggs. They are transluscent white in colour and are difficult to make out when freshly laid. The host caterpillar invariably recovers consciousness after egg-laying is over and moves about carrying the eggs; it lives till it is finally consumed by the feeding parasite grubs. In such movements the eggs and the grubs of the parasite are partially shed.

The eggs hatch in 56–72 hours and the grubs start feeding immediately from the same place and never leave their positions. Soon they swell into light amber-colored vesicular bodies and get the salmon-pink colour afterwards. Each grub spins a cocoon after full development and pupates therein; the cocoon is white except when spun in cotton bolls when it turns light chestnut brown. The adult emerges from the cocoon in about seven days by biting a hole in it. Emergence of males first was not the feature as recorded by Sweetman (1936) nor mating observed inside the female cocoons. Parthenogenesis is fairly common. The life-cycle is completed in 12–15 days.

In nature, the bethylids have been observed to parasitize other hosts like *Antigastra* and *Adisura* caterpillars.

2. *Apanteles pectinophoræ*.—This internal parasite emerges from the host after it has cocooned. The parasitic grub comes out of the body of the host and spins a white solitary cocoon within that of the host. Only a single parasite issues from a cocoon. It is not possible by external symptoms to say whether a particular caterpillar is parasitized or not. The parasite is in evidence right through from December to June though not in appreciable numbers as will be seen from the Tables II and V. It appears to be an exclusive parasite of the pink bollworm.

3. *Microbracon gelechidiphagus* Ramk.—This occurs as an exclusive parasite of the advanced caterpillar stages of the pink bollworm infesting the medium and large-sized bolls of cotton. It is easily distinguishable from the other two Microbracons by the lightness of the body color and the two faint fuscous patches on the second and third abdominal segments of the female. Under the field conditions the paralysed caterpillars are uniformly found deep inside the boll, and hence it is to be presumed that the parasites reach the host through any exit hole present in it; otherwise it is very difficult for this small insect to reach the host from outside with its ovipositor. Under laboratory conditions, however, it is able to parasitize the pink bollworm when given in tender bolls just as the other two species do. Adults have also been reared from other hosts like that of *Earias fabia* and *E. insulana* and they are found to conform to the color pattern of the parent. The incidence of field parasitization rarely exceeds 22·5% [Tables IV and VI]. The insect appears very late in the season when the host population is generally high. There is no material difference in the duration of the life-cycle between this and the other species.

TABLE IV

Incidence of Microbracon gelechidiphagus *Ramk. in the* Platyedra gossypiella *caterpillars in the shed buds and bolls of cotton (Cambodia)*

Year and Month	Buds and Flowers				Bolls			
	Total picked	Host population	No. parasitized	Percentage of parasitization	Total picked	Total population	No. parasitized	Percentage of parasitization
1938								
November II ..	679	4	..	..	313	3	..	..
December I ..	4,203	17	..	..	6,597	50	..	..
Do. II ..	2,373	10	..	..	18,483	100	..	..
1939								
January I ..	2,154	1	..	..	11,993	33	..	..
Do. II ..	114	..	..	..	2,386	14	..	..
May I ..	1,507	136	11	8·8	710	310	69	22·25
Do. II ..	7,917	151	5	3·31	1,597	128	1	0·83
June I ..	2,972	60	..	..	259	68	1	1·47
December II ..	1,563	25	..	..	1,037	10	..	..
1940								
Januray I ..	7,028	83	..	..	4,963	48	..	..
Do. II ..	5,910	35	..	..	3,687	21	..	..
February I ..	752	3	..	..	794	10	..	..
July II ..	560	117	..	..	407	353	7	1·98
August I ..	228	28	..	..	109	93	..	..

Note.—I and II refer to the first and second fortnights respectively.

TABLE V

Emergence record of parasites from the shed buds and bolls of cotton 600 *each picked on* 22*nd July* 1940

Date of Emergence		Nature of Parasites			
		Microbracon gelechidiphagus	*Apanteles pectimophoræ*	*Goniozus* n. sp.	*Elasmus johnstoni*
1940					
July	23	5	11	19	..
	26	6	12	15	2
	27	16	5	2	..
	28	6	4	2	..
	29	4	3	2	..
	30	3	..	3	..
	31	..	3	..	..
August	1	..	1	..	..
	2	..	1	..	..
	3	..	4	..	..
	4	..	9	..	..
	5	..	..	..	..
	9	..	5	..	..
	10	..	3	..	..
	12	4	3	..	..
	13	1	6	1	..
	14	..	..	..	..

Note.—M. lefroyi, Bassus, Actia and *M. greeni* were not recovered during the period.

TABLE VI

Incidence of Microbracon gelechidiphagus *in the* Platyedra *caterpillars in the standing large-sized bolls of Cambodia for the years* 1933 *and* 1934

Year and Month	Host population	Number affected by parasites	Percentage of parasitization
1933			
January	65	..	..
February	63	..	..
March	163	..	..
April	26	2	7·69
May	422	39	9·24
June	575	41	7·10
July	857	28	3·26
1934			
January	..	..	..
February	115	2	1·60
March	173	1	0·58
April	184	1	0·54
May	170	2	1·18

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CYTO-MORPHOLOGICAL FEATURES OF *LIMNANTHEMUM CRISTATUM* GRISEB. AND *ENICOSTEMMA LITTORALE* BLUME.

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I. Introduction

Embryological and morphological investigations in Gentianaceæ cannot be said to be meagre. The earliest reference to this family is found in the great work of Hofmeister (1858). The genus *Cotylanthera* has been investigated by Figdor (1897) and Sussenguth (1927). Guerin (1903, 1924, 1925 & 1926) gave a detailed account of the pollen development in *Gentiana*, *Swertia*, *Erythrea*, *Chlora*, *Cicendia*, *Menyanthes* and *Limnanthemum*. The other important work that should be made mention of, is that of Stolt (1921), who investigated fourteen members of this family, of wnich ten belong to the genus

Gentiana. The remaining four plants were *Erythrea centaurium, Exacum affine, Chlora perfoliata* and *Halenia elliptica.* He has also studied some members of the sub-family Menyantheoideæ, *Limnanthemum nymphoides, Menyanthes trifoliata* and *Villarsia reniformis.* He has discussed the systematic position of Gentianaceæ and the division of the natural order into two sub-families, taking the manner of endosperm formation as the criterion.

After Stolt's work there is not any work on this family worth mentioning. Through the kindness of Dr. Sampathkumaran, the unpublished work of one of his research students (Vyasaraja Rao) was made available to me. In that work a comparative account of some representative species distributed over five genera is given. In this paper, the development of micro- and macrogametophytes and the embryo in *Enicostemma littorale* Blume., is described. An account of the floral ontogeny in *Limnanthemum cristatum* Griseb., is given. Some details of embryo-sac development and microsporogenesis are also recorded. The chromosome numbers of the above said two species have also been determined for the first time.

II. Materials and Methods

Enicostemma littorale Blume., is a perennial weed, four inches or more in height. Leaves are linear, oblong, three-nerved at base, one to two inches long and glabrous. Flowers sessile in clusters along the stem. Corolla white, $\frac{1}{4}''$ long. Capsules ellipsoid with many seeds.

Limnanthemum cristatum Griseb., is a member of the sub-family Menyantheoiedæ. It is an aquatic herb with deeply cordate entire and shortly petioled leaves. Stems are long floating in water and having a single leaf at the end (node). Flowers are numerous in dense clusters. Calyx divided almost to the base, Corolla white, lobes being obovate with a longitudinal crest along the middle of each lobe.

For cytological studies, root-tips and flower buds were fixed, the latter after acetocarmine examination, in Müntzing's modification of Navaschin's fluid. Corrosive sublimate fixative and formalin-acetic-alcohol were used for morphological work. Materials were embedded in paraffin-wax and sections were taken at thicknesses varying from 6 to 15 microns. The sections were stained in iron-alum-hæmatoxylin, Ehrilch's hæmatoxylin and Newton's iodine gentian-violet.

III. Limnanthemum cristatum Griseb.

(*a*) *Organogeny of the flower.*—The organs of the flower arise in acropetal succession, *i.e.*, sepals, petals, stamens and carpels. The primordium of the flower arises as a knob-like protuberance in the axils of the bracts.

This structure at a later stage becomes broadened at the apical portion and the primordia of the sepals arise from the edges of this disc-like portion. Immediately following the origin of the sepals, the petals also arise (Fig. 1). The petals and the sepals grow for some time without the initiation of the anthers (Fig. 2). Later the anthers also originate (Fig. 3). The gynœceum is differentiated at a still later stage (Fig. 4). Such an acropetal succession of floral organs is not uncommon though the usual order is calyx, stamens, corolla and pistil. The latter said type of floral ontogeny has been recorded in many species distributed over various families, as examples of which may be cited, *Asteracantha* (Rangaswamy, 1941), *Angelonia* (Srinivasan, 1941) and Scrophularia (Schertz, 1919). The type of development as is met with in the present case has been recorded in *Cleome Chelidonii* (Raghavan, 1937) and in *Cratæva* (Raghavan and Venkatasubban, 1941).

(*b*) *Development of the microsporangium.*—The archesporium of the anther is hypodermal (Figs. 5 & 6). it consists of a band of cells which through furtner divisions give rise to the wall layers and the sporogenous tissue. The mature anther at the stage just before the pollen mother cells enter upon the meiotic stages, has a wall made up of five layers, the innermost of which functions as the tapetum (Fig. 7). The tapetum is uni-nucleate to begin with, but soon undergoes repeated mitotic divisions and thus becomes multi-nucleate. Immediately following this multi-nucleate condition nuclear fusion takes place, resulting in nuclei with more than one nucleolus. Stages of fusion of nuclei are shown in Fig. 7. Such a fusion has recently been recorded in many angiospermous plants and its possible significance has been discussed at length in a previous paper on *Portulaca* (Raghavan and Srinivasan, A. R., 1941 *c*), where also such a phenomenon was met with. The tapetal cells degenerate after the formation of tetrads.

(*c*) *Somatic chromosomes.*—Eighteen chromosomes were found in somatic metaphase plates of this plant (Fig. 8). Except two chromosomes, which are long and have sub-median constrictions, all the others are comparatively short. The constrictions in these short chromosomes are terminal or sub-terminal.

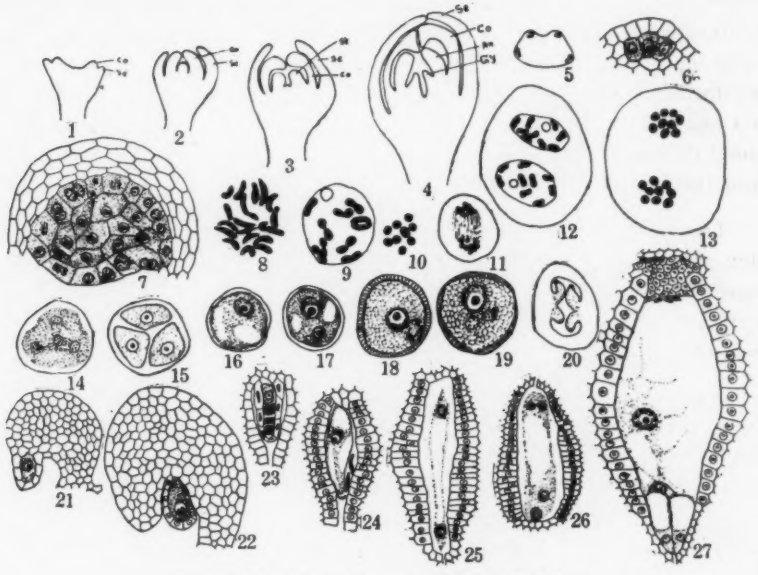
(*d*) *Development of the microspore.*—During diakinesis nine bivalents were observed in the pollen mother cells. Except one all the other bivalents are of the rod-type and possess each a single terminal chiasma. This terminal chiasma formation has been reported to be characteristic of all small chromosomes (Lawrence, 1931). The observations of Nandi (1936) also go to confirm this view. In the present case there are only two long chromosomes, which presumably form a ring bivalent with two terminal

chiasmata. All the other somatic chromosomes which are smaller, form only rod-bivalents. During early diakinesis stages, the bivalents are distributed along the periphery, equidistant from each other. Gates (1909) first observed this uniform spacing of bivalents in *Oenothera* and suggested that this was due to a mutual repulsion of bivalents. In the later diakinesis stages as is represented in Fig. 9, this repulsive force diminishes and the bivalents move away from the periphery towards the centre.

The nuclear membrane and the nucleolus disappear and the nucleus after a short prometaphase enters upon the first metaphase. In the metaphase all the nine bivalents are arranged in the equatorial plate (Fig. 10). In the polar view one of the bivalents appears to be larger than the others. Obviously this is the ring-bivalent met with during diakinesis.

Anaphase I is regular and due to the disjunction of the bivalents nine chromosomes migrate towards each of the two poles (Fig. 11). The first telophase nucleus is constructed from the flat anaphase plate and therefore it is at first nearly flat. First telophase is succeeded by interphase almost immediately. There is practically no difference between the telophase I and the early interphase. During interphase the nucleus expands and assumes a globular appearance, while at telophase it was flat. At the same time the chromosomes spread around the periphery and simulate diakinesis stage. Possibly there is some movement of the chromosomes relative to one another. The split of the chromosomes becomes more obvious towards the end of interphase (Fig. 12). But for these two differences, the split appearance of the chromosomes and the globular nature of the nuclei, there is nothing else to differentiate the interphase from the telophase nuclei. As the interphase advances, the nuclear membrane disappears and the second prometaphase follows. During this the chromosomes move towards one another and ultimately the second metaphase results (Fig. 13). Both the plates are flat and nine chromosomes are found in each group. The second division takes place normally. During the second telophase four additional spindles arise connecting all the telophase groups with each other. The telophase groups are arranged in the tetrahedral fashion (Fig. 14).

The uni-nucleate pollen grains break apart (Fig. 15). At first these have an irregular outline but soon assume a rounded configuration. Each grain has dense protoplasm and a large nucleus. Vacuoles are also present. The uni-nucleate pollen grain grows in size and a large vacuole appears in the cell towards one side. There is a nucleus embedded in the protoplasm (Fig. 16). This is now in a resting condition. The pollen grain continues to grow in size during which time the nucleus enlarges and begins to divide. This



FIGS. 1–27. *Limnanthemum cristatum* Griseb.

FIGS. 1–4. Stages of floral ontogeny. × 750. FIG. 5. Transverse section through a young anther. × 220. FIG. 6. Archesporium of the anther. × 1100. FIG. 7. Section through a mature anther showing the wall layers, tapetum and the mitotic division in the tapetal cells. × 750. FIG. 8. Somatic chromosomes. × 3900. FIGS. 9 and 10. Diakinesis and metaphase I. × 3900. FIG. 11. Anaphase I. × 1500. FIG. 12. Interphase. × 3900. FIG. 13. Second metaphase. × 3900. FIGS. 14 and 15. Second telophase and tetrad formation. × 1500. FIGS. 16–19. Stages of pollen development. × 1500. FIG. 20. Transverse section through a young ovary showing the unilocular ovary with two parietal placentas. × 150. FIG. 21. Archesporium of the ovule. × 750. FIG. 22. Ovule at the megaspore mother-cell stage. × 750. FIG. 23. Linear tetrad. × 750. FIGS. 23–27. Embryo-sac stages. All except Fig. 26 are of magnification × 750; Fig. 27 × 350.

division is a very rapid one and stages are not frequently found. As division proceeds, the protoplasm begins to fill once more the interior of the cell. The two daughter nuclei resulting from this division pass to a resting condition (Fig. 17). The two nuclei both with prominent nucleoli always show a big difference in size. The larger one which occupies a more central position in the pollen grain is the tube-nucleus. The smaller one is the generative nucleus and is about one-third the size of the tube-nucleus. The generative cell is elongated usually with curved pointed ends, but is always circular in cross-section. Around the generative nucleus is a well-defined generative cell. The generative nucleus undergoes another division resulting in two small daughter nuclei. These two nuclei are those of the male cells. Fig. 18 shows the anaphase of the division of the generative nucleus.

The mature pollen is thus three celled consisting of a large tube cell and two male cells (Fig. 19). There are three germinal pores. The cytoplasm of the mature pollen grain is much vacuolate.

(*e*) *Megasporogenesis.*—The ovary is bicarpellary and unilocular (Fig. 20). It has got two parietal placentas each bearing two rows of ovules. The ovules arise as protuberances which at later stages lengthen and become much curved. The archesporium of the ovule is differentiated hypodermally as a single cell at the tip of this curved ovule (Fig. 21). The single integument is differentiated shortly after this stage and its growth is so rapid that at the stage wnen the archesporium has elongated into the megaspore mother cell, the ovule becomes a massive structure. The ovule becomes anatropous at this stage (Fig. 22) The megaspore mother cell is surrounded on all the three sides except the chalazal end by the nucellus. The nucellus as in all sympetalous families, is so very much reduced that it is nothing more than the single epidermal layer surounding the embryo-sac mother cell. This nucellar layer is short-lived. It is crushed between the developing megaspore mother cell and the integument and finally degenerates.

The nucleus of the megaspore mother cell undergoes the usual meiotic divisions and gives rise to a linear tetrad of megaspores, of which the chalazal megaspore functions (Fig. 23). At this stage the nuclei of the nucellar cells degenerate and the nucellar cells themselves become much distorted in appearance. Due to the growth of the megaspore into the uni-nucleate embryo-sac, the nucellar layer disintegrates completely causing the embryo-sac to come into direct contact with the innermost layer of the integument (Fig. 24).

The cells of the innermost layer of the integument become radially elongated and enriched in cytoplasmic contents. The layer which is in contact with the embryo-sac is the tapetum and it has probably a nutritive function. Unlike the tapetum of the anther the integumentary tapetum does not occur in all plants. It is characteristic of many sympetalous families as examples of which may be mentioned, Solanaceæ (Bhaduri, 1935), Scrophulariaceæ (Srinivasan, 1940), Labiateæ (Billings, 1909), Lobeliaceæ (Kausik, 1935) and Orobancheæ (Srivastava, 1939). The tapetum in the present case does not surround the whole embryo-sac. It surrounds the major portion of the embryo-sac leaving only ordinary cells at the micropylar region. In *Nicotiana glutinosa* (Raghavan and Srinivasan, A. R., 1941 *a*) and in some of the members of Scrophulariaceæ (Srinivasan, 1940), the tapetal cells were found to be aosent in both the chalazal and the micropylar ends of the embryo-sac. In the present case the tapetal cells were

uni-nucleate. This uni-nucleate condition is found to be usual. Exceptions also occur as in the cases of *Solanum melongena* (Bhaduri, 1932) and *Orobanche ægyptiaca* (Srivastava, 1939), where the tapetal cells were found to be bi-nucleate.

Through the division of the single nucleus of the uni-nucleate embryo-sac and the migration of the daughter nuclei to opposite poles, the bi-nucleate embryo-sac is formed (Fig. 25). One more division results in the four-nucleate embryo-sac (Fig. 26), from which the eight-nucleate embryo-sac is formed.

The mature embryo-sac is large and consists of the synergids, the egg-cell, the secondary nucleus and the degenerated antipodals. The synergids are large with prominent basal vacuoles (Fig. 27). The secondary fusion nucleus also is big in size. The antipodals are extremely ephemeral. In the mature embryo-sac the tapetum extends beyond the antipodal end of the embryo-sac and thus encloses within its limits part of the chalazal tissue. The cells of this tissue are devoid of their cytoplasmic contents and their nuclei present a degenerated appearance. Probably their contents are absorbed by the tapetal cells forming the jacket-like structure. These tapetal cells adjacent to this chalazal tissue take on a deeper stain than the rest. This probably supports the above-mentioned surmise. Such a case has been previously reported in *Lobelia trigona* by Kausik (1935), who is also of the opinion that it is a nutritive tissue from which the adjacent tapetal cells absorb food-materials.

IV. Enicostemma littorale Blume.

(*a*) *Development of the anther.*—Three cells situated in the form of a hypodermal plate at each of the four corners of the young anther, constitute the archesporium of the microsporangium (Fig. 28). These cells are longer than the surrounding cells with dense protoplasm and prominent nuclei (Fig. 29). Hence the archesporium is not very extensive. The lobes of the anther become more and more conspicuous after the initiation of the archesporium. Through division of these cells, the primary wall layer and the primary sporogenous layer are formed. The primary sporogenous layer gives rise to one more sporogenous layer (Fig. 30). The periclinal divisions of the cells composing the primary parietal layer (Fig. 30) result in the formation of more wall layers. In the mature anther there are two to three layers of sporogenous cells surrounded by four wall layers, the innermost of which forms the tapetum of the anther (Fig. 31). The tapetum is in close contact with the outermost layer of the sporogenous cells. One interesting feature is that vacuolation proceeds rather very early in the tapetal cells. Even during the

resting stage of the nuclei of the pollen mother cells, each tapetal cell shows a single large vacuole, the nucleus being located peripherally on the side of the cell towards the wall layers. Another important fact is that the tapetal cells remain uni-nucleate even at the time of tetrad formation, after which they finally degenerate. Such a uni-nucleate condition of the tapetum, though not very common, seems to be of wide occurrence in the family Leguminaceæ. It has been reported in *Neptunia oleracea* (Singh and Srivapuri, 1935), *Albizzia lebbek* (Maheshwari, 1931), *Lathyrus odoratus* (Latter, 1926), *Medicago* spp. (Reeves, 1930) and *Cassia didymobotrya* (Sethi, 1930).

It is also of interest to record here that what is usually regarded as a relatively important morphological feature, should show a fundamental variation in two plants belonging to the same family, *Enicostemma* and *Limnanthemum*. In the latter plurinucleate cells were met with, while in the former uni-nucleate cells are present. This only goes to support the view given expression to in a previous paper (Raghavan and Srinivasan, A. R., 1941*c*), while discussing the relative importance of these morphological details. There is none that is too minor to recount and it is only after a critical study of a particular group that we can know, whether a particular feature is characteristic or not. Apparently important ones do not often have this significance, while what may be regarded as trivial details not infrequently are found to disclose importance from this point of view.

During meiosis of the pollen mother cells nineteen bivalents were observed at first metaphase (Fig. 32). So the haploid chromosome number of this plant is 19. The bivalents are fairly large. The rest of the meiosis takes place normally.

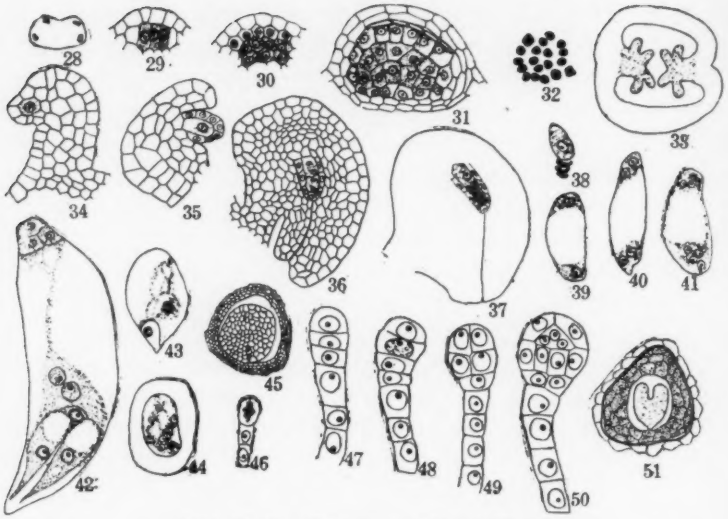
(*b*) *The ovary and the ovule.*—The ovary is bicarpellary and unilocular. There are two parietal placentas each bearing 4 to 6 rows of ovules (Fig. 33). The ovules first arise as straight protuberances. Later the tip of the ovule in which the archesporium is differentiated, becomes bent at right angles to the body of the ovule (Fig. 34). The single integument is differentiated just from the base of this tip and grows rapidly, so that during dyad stages, it grows past the dyad forming a narrow micropylar canal (Fig. 36). There is a single layer of nucellus composed of 7 to 8 cells surrounding the megaspore mother cell, which degenerates at a later stage. The mature ovule is anatropous.

(*c*) *Megasporogenesis.*—The archesporium of the ovule is a hypodermal cell which elongates and directly functions as the megaspore mother cell without cutting off any parietal cells (Figs. 34 & 35). The megaspore mother cell undergoes meiosis and gives rise to the dyad (Fig. 36). The nuclei of the dyad cells divide homotypically and the plane of their division is not

straight (Fig. 36). Ultimately a linear tetrad is formed (Fig. 37). By this time the cells of the nucellus are crushed between the developing tetrad and the growing integument and are distorted in appearance. They degenerate when the chalazal megaspore develops into the uni-nucleate embryo-sac at the expense of the micropylar cells which degenerate (Fig. 38).

The uni-nucleate embryo-sac is full of cytoplasm and soon the bi-nucleate embryo-sac is formed (Fig. 39). From tnis the four-nucleate embryo-sac is formed (Fig. 40). Through the division of the four nuclei, the eight-nucleate embryo-sac is formed. Thus the development of embryo-sac in *Enicostemma* conforms to the normal monosporic type of embryo-sac development (Maheshwari, 1937). In the eight-nucleate embryo-sac, the four nuclei at the micropylar end are somewhat larger than those at the chalazal end (Fig. 41). In Fig. 41 one of the four nuclei at the micropylar end is seen to start towards the centre. This nucleus and another from the antipodal end constitute the polar nuclei, and these after fusion give rise to the secondary nucleus. The remaining three nuclei at the micropylar end organise the egg-apparatus. The synergids are long with accuminate tips, and large basal vacuoles in the mature embryo-sac (Fig. 42). Their ends lie apart from each other during early stages. The egg-cell lies in the centre at the base of the synergids. The polar nuclei which have not yet fused lie in the neighbourhood of the egg-cell. The three nuclei at the chalazal end form the antipodal cells which are more prominent than in the case of *Limnanthemum*. A comparative study of the antipodals of the Gentianaceæ is interesting. Stolt (1921) classified them into various groups and sub-groups based on their number and formation. The main types are (1) three antipodals, normally-developed; (2) three, many nucleate and very well-developed; (3) three or more, poorly-developed and (4) always more than four and strongly developed. The antipodals of *Enicostemma* come under the first mentioned type, while those of *Limnanthemum* belong to the third type. A similar variety in the structure of antipodals has been met with in Rubiaceæ and Fagerlind (1937) has classified them according to their nature. In that family the antipodals of *Spermacoce* on account of their size and nature of arrangement, have been pointed out as showing a tendency towards formation of antipodal haustoria (Raghavan and Srinivasan, A. R., 1941 *b*). As in Gentianaceæ a gradation from the ephemeral antipodals, to structures made up of numerous strongly developed antipodal cells, is found in Rubiaceæ also. The whole embryo-sac is a bit curved and slender.

(*d*) *Endosperm and embryo.*—After fertilization the embryo-sac becomes laterally extended and presents an ellipsoid appearance (Fig. 43). The endosperm nucleus divides (Fig. 43) and gives rise to the nuclear endosperm



FIGS. 28–51. *Enicostemma littorale* Blume.

FIG. 28. T. S. through an young anther. × 150. FIGS. 29–31. Stages of anther development. × 750. FIG. 32. M. I plate showing 19 bivalents. × 3900. FIG. 33. T. S. through a young ovary. × 180. FIGS. 34–38. Stages of megasporogenesis. × 750. FIGS. 38–41. Embryo-sac stages. × 750. FIG. 42. Mature embryo-sac. × 2500. FIGS. 44 and 45. Ovules showing nuclear and cellular endosperm. × 150. FIG. 46. Three-celled pro-embryo, apical cell in division. × 750. FIGS. 47–50. Embryo stages. × 900. FIG. 51. Section through a young seed. × 150.

(Fig. 44). The endosperm becomes cellular at a comparatively early stage. When a linear pro-embryo of three cells is formed, the ovules show cellular endosperm surrounding the pro-embryo (Fig. 45). The endosperm tissue during earlier stages, is not in contact with the integumental tissue. Thus a space is present which, however, is filled up during later stages owing to increase both in the size and number of endosperm cells.

Through the periclinal divisions of the oospore, a linear three-celled pro-embryo is formed in which the apical cell undergoes periclinal division (Fig. 46). Ultimately a six-celled pro-embryo is formed (Fig. 47). When the pro-embryo is made up of seven cells (Fig. 48), the octant is organised by the anticlinal division of the two apical cells (Fig. 49). The suspensor at this stage consists of five cells, that nearest to the octant elongating tangentially and forming the hypophysis. The hypophysis divides at a later stage and contributes to the organization of the dermatogen (Fig. 50).

Fig. 51 shows a transverse section through a young seed. There is an young embryo in which lobing of the cotyledons has been completed. There

are two to three layers of large endosperm cells. The endosperm cells in the immediate neighbourhood of the embryo, degenerate creating a lacuna round the embryo. Starch grains and other inclusions are found in the endosperm cells. The seed would appear to have a seed coat composed of only a single layer of cells. Presumably all the other layers of the integument degenerate during the conversion of the ovule into the seed. Even this layer presents a very disintegrated appearance. However protection seems to be given to the embryo and the surrounding endosperm by the thickened internal wall of this layer.

V. Discussion

Many families of the Sympetalæ are characterised by the occurrence of special devices for the nutrition of the embryo-sac. Roughly these devices can be classified into two types: (i) the formation of tissues which are nutritive by themselves, and (ii) development of tissues which form as it were, a medium through which the food materials contained in the integumental tissue is absorbed, stored up and ultimately transferred to the embryo-sac.

Considering the tapetal tissue in this light, the integumentary tapetum present in many of the families of Sympetalæ, seems to have a two-fold function. A comparison may be drawn with the tapetal tissue present in the microsporangium. There, the tapetum is nutritive in function. It undergoes Karyological changes such as the multiplication of the nuclei and their ulitmate fusion. All these changes are probably intended to enrich the cells in food contents. Moreover, soon after tetrad formation, the tapetum degenerates, its contents being used up by the pollen mother cells during their meiotic activity. Thus as soon as the need for the nutrition of the pollen mother cells is over, the tapetum of the anther degenerates. In the case of the integumentary tapetum however, it does not degenerate until late in the life-history of the endosperm. Very probably during the initial stages, the tapetum of the ovule is nutritive in function and supplies its food contents to the developing embryo-sac. At the same time, its other function seems to be to absorb food materials from the surrounding integumentary tissue and pass it on to the endosperm tissue, upon a portion of which the developing embryo feeds. Thus, it has a dual function, (1) that of directly contributing nutrition during the prefertilisation stages and (2) of a haustorial nature, to absorb food materials from the integumental tissue, and transfer it to the endosperm during post-fertilization stages.

The chalazal tissue is, in many cases, found to be nutritive in function, its contents being absorbed by haustoria of various kinds and made available to the developing embryo. Such haustorial structures always arise in the post-fertilization stages, whatever their origin may be. These haustoria

are named after the regions from which they take their origin. For instance, antipodal haustoria are developed through the division of the antipodal cells and their extension into the chalazal regions, *e.g.*, some members of Rubiaceæ (Fagerlind, 1937). The endosperm haustoria take their origin from the endosperm nucleus and are termed micropylar or chalazal according to their position, *e.g.*, members of Scrophulariaceæ (Srinivasan, 1940). Suspensor cells develop and give rise to the suspensor haustoria, *e.g.*, Acanthaceæ (Rangaswamy, 1941) and Rubiaceæ (Fagerlind, 1937).

All classes of haustorium formation take place in the post-fertilization stages. So the chalazal tissues into which these haustoria penetrate, assume a nutritive role in the economy of the ovule only during post-fertilization stages. In *Limnanthemum* there is no haustorium reported (Stolt, 1921). But a part of the chalazal tissue assumes a nutritive function during pre-fertilization stages only. In *Lobelia trigona* (Kausik, 1935) this tissue assumes nutritive role both in the pre- and post-fertilization stages. In the pre-fertilization stages the tissue is enclosed within the limits of the integumentary tapetum which extends beyond the antipodal end of the embryo-sac. The tapetal cells composing this jacket-like structure " absorb materials from the chalazal nutritive tissue which they enclose and supply the growing embryo-sac with extra nutrition". Such a case is now found to occur in *Limnanthemum* also. The essential difference between the two cases lies in the absence of endosperm haustoria in *Limnanthemum*. Due to the formation of endosperm haustoria in *Lobelia*, the chalazal tissue assumes a nutritive role during the post-fertilization stages also, while in *Limnanthemum* the nutritive role of the chalazal tissue is restricted to pre-fertilization stages as no haustorial structures are formed.

Considering the various nutritive devices described above, it is clear that all the haustorial devices belong to the second category of nutritive devices mentioned at the beginning. The integumentary tapetum belongs partly to the first type (as it functions directly as a nutritive tissue in the pre-fertilization stages), and partly to the second category (because it serves as a medium for transportation of the food from the integumental tissues to the endosperm during post-fertilization stages. The chalazal nutritive tissue,—irrespective of whether it becomes nutritive at only one period of its life-history as in *Limnanthemum*, or assumes a nutritive rôle at two definite periods as in *Lobelia*,—is of a directly nutritive nature and hence belongs to the first type.

VI. Summary

The organs of the flower of *Limnanthemum cristatum* Griseb., arise in acropetal succession.

The tapetum of the microsporangium is multi-nucleate and shows nuclear fusion.

The diploid number of the species is determined for the first time as 18 and is confirmed from the results of meiotic counts ($n = 9$). The pollen grains are three-celled at the shedding time.

The embryo-sac shows integumentary tapetum and a chalazal nutritive tissue. Their role in the economy of the nutrition of the ovule is discussed.

The development of microsporangium in *Enicostemma littorale* Blume., is normal. The tapetum is uni-nucleate. The haploid chromosome number of this species has been recorded for the first time as nineteen.

The development of embryo-sac and embryo are of the normal type.

VII. Acknowledgement

The work was carried out in the Botanical Laboratory of the Annamalai University. It is a source of sincere pleasure to record my indebtedness to Dr. T. S. Raghavan, M.A., Ph.D. (Lond.), F.L.S., Professor and Head of the Department of Botany, under whose guidance this investigation was made.

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ON THE EMBRYOLOGY OF *SQUILLA*

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1. Introduction

More than sixty years have passed since the details of the complicated metamorphosis of the Stomatopoda have been brought to light by Claus and still our knowledge of this interesting group is far from complete. This is especially so with regard to the early embryonic development and in the large volume of work done in recent years on the embryology of the Crustacea the gap so left is conspicuous. Indeed, except for a short paper by Komai (1924) on the changes in the external form that precede hatching in *Squilla oratoria* I am not aware of the existence of any other work on the early development of the Stomatopoda. "This seems to be rather a striking fact," writes Komai, "because, first, among the whole of the Malacostraca, only the rare and peculiar Anaspidacea shared with that order such a situation, second, the animal belonging to that order is by no means uncommon in any part of the tropical, subtropical and temperate seas in the world, and third, the larval stages have been familiar to us for a fairly long time." Komai's

contribution, no doubt, has been of great value, but the statement is applicable even now. Moreover our knowledge of the development of many of the Malacostraca has increased considerably since then and rare forms like *Anaspides* have also been investigated (Hickman, 1937). Hence it was thought that a study of the embryology of *Squilla* would be of interest and the present communication embodies the results of investigations in this direction.

It is a pleasure to record here my sincere thanks to Professor R. Gopala Aiyar, Director, University Zoological Research Laboratory, Madras, for all the encouragement and help which he has given me during the course of the work. My thanks are also due to the University of Madras for awarding me a Research Fellowship.

2. *Material and Methods*

Four species of *Squilla* are common on the Madras Coast. These are *S. holoschista* Wood-Mason, *S. nepa* Latreille (Bigelow), *S. raphidea* Fabricius and *S. wood-masoni* Kemp. They are often brought up in fishermen's nets and are sold along with prawns and crabs in the local markets. Fishermen were given instructions to bring pregnant and berried female specimens to the laboratory in a living condition, but such catches were not frequent. Many specimens of *S. wood-masoni* and *S. raphidea* carrying eggs in an advanced stage of development were obtained, but none belonging to the other species. Among the large number of specimens of *S. holoschista* and *S. nepa* brought to the laboratory even pregnant females were very few. Hence it was decided to concentrate attention on the two former species. Of these *S. raphidea* is the larger in size—in fact it is the largest of the four species mentioned above. The eggs are correspondingly bigger and more numerous. It also shows less pigment in the yolk. The berried females were kept alive in the sea-water tanks and eggs were removed from the mother in small masses at intervals and were fixed for sectioning or for making whole mounts. Though the animals survived in a fairly healthy condition for weeks they cast away the egg masses from their hold after a day or two and refused to carry them even when induced to do so. This was unfortunate for, the rejected egg masses, when kept separately in dishes, became very quickly subject to the attack of ciliate parasites and did not develop. In spite of every possible precaution such as filtering the sea water before use, keeping the water in motion with the aid of plunger jars, etc., the attempt to check the attack of parasites failed and the eggs could not be developed outside the hold of the mother. Thus there was no other way to pursue the study, but to wait for and collect the stages from the berried specimens fresh from the sea. Some of the later stages of *S. raphidea* and a few of *S. wood-masoni*

were obtained in this manner. But in spite of careful and exhaustive search the earliest stages could not be obtained by this means and so it was decided to keep pregnant female specimens in the laboratory and see if they would lay eggs in captivity. Several large females of the two species with ripe ovaries were kept under observation. These specimens were usually obtained with their mates and in the sea-water tanks the couples were kept unseparated. They lived healthily, but even after weeks the eggs were not extruded. The ripe ovary could be seen filling the whole body cavity on the dorsal side. The water in which they were kept was then aerated and kept in motion with the aid of a stirrer attached to the axle of a small table fan from which the blades had been removed. The tip of the stirrer rotated just below the surface of the water and in doing so, it regularly sucked in quantities of air which were scattered and sent in very tiny bubbles throughout the volume of water. The animals were prevented from coming in contact with the rapidly revolving stirring rod and injuring themselves by a piece of wire netting. This proved to be very efficient in keeping the animals healthy and active and in such a medium they extruded the eggs in a short time.

The process of extrusion was observed on three occasions, twice in *S. raphidea* and once in *S. wood-masoni*. In both, the process is similar. The animal lies on its back, turned a little towards one side, the telson and uropods bent inwards. The egg mass gently oozes out of the genital aperture in a thin flat continuous stream. The pleopods work slowly and the extruded eggs crowd together in a mass. The whole mass and especially the stream that freshly comes out of the genital aperture is extremely sticky. The eggs of *S. raphidea* are golden yellow in colour and those of *S. wood-masoni* deep brick-red. Except the regular beat of the pleopods the animal shows no other movement. The eggs come out and form into a mass. It takes nearly 100 minutes for the whole egg mass to be extruded in the case of *S. raphidea* and a little less in *S. wood-masoni*. After extrusion the mother turns and swims a little. She comes back immediately and spreading her thoracic appendages over the egg mass, collects it in her posterior maxillipeds. The whole mass is rolled over again to make it compact and is held tight against the ventral surface of the body. In all the three cases extrusion took place in the morning before 9 O'clock and the animals were not observed to moult before the process.

After carrying the egg mass about for a few hours the animal drops it. Every method was tried to induce to take it up but, in vain. It takes it up at times, but only to throw it farther away. Occasionally the mother was seen to eat the eggs. It was not possible to develop these eggs also separately due to the attack of coliate parasites.

Each egg is rounded in shape and possesses a tight fitting envelope. When just coming out of the body they are seen to be embedded in a jelly-like substance which sticks them together. The embryos of the berried females brought from the sea, on the other hand, are arranged in a thin expansive mat-like cluster. In the case of undisturbed specimens the whole cluster appears as one continuous unbroken sheet. The embryos lie two or occasionally three deep. The envelope of each embryo is attached to those of the surrounding ones by means of short cylindrical threads three or four in number.

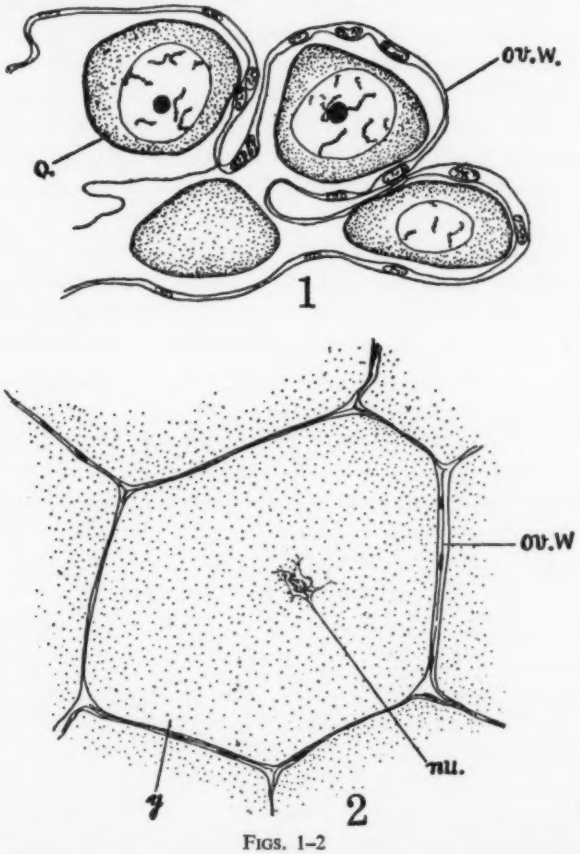
Eggs and embryos were fixed in Smith's fluid for 48 hours. The fixative was changed twice and the material after fixation was stored in 5% formalin in the dark. For embedding it was cleared first in methyl salicylate from 90% alcohol and then in cedar-wood oil. Paraffin of 58° C. M. P. was used. About 45 minutes impregnation was given in the bath. Orientation was possible, but it was found to be easier to cut a large number of embryos in a mass and to pick out the series that were in the required plane. Sections were cut at 8 microns. There was slight fracturing of the yolk, particularly in the younger stages, but several good series were obtained. Sections were stained in Heidenhain's iron hæmatoxylin. Counterstaining was found to be extremely difficult, the cells refusing to take up any of the common acidic dyes. Finally prolonged immersion in orange G gave somewhat satisfactory results. In making whole mounts embryos were fixed in 10% formalin and after removing the covering membrane, were stained in Delafield's hæmatoxylin. But the preparations were not very clear and hence of little use.

The development of the two species, *S. raphidea* and *S. wood-masoni*, takes place in identical manner and since no continuous series of stages of either form has been obtained, the following account of the development, as far as it has been possible to follow, is complete only when the two species are taken together. Except when describing the general trend of development, the material from which observation has been made will, however, be specified.

3. The Growth of the Egg before Extrusion

The eggs lie within the folds of the ovarian wall, which, when the eggs are small, appears fairly thick with rounded nuclei arranged in single file. In the young stages each egg consists of a rounded mass of protoplasm which stains blue with hæmatoxylin. At the centre of it lies the large germinal vesicle, more than half the size of the whole egg. It remains unstained and clear and shows a conspicuous nucleolus at the centre (Fig. 1). The nucleolus is circular in shape and dense blue in colour. The cytoplasm does not show any inclusions in the early stages and stains homogeneously. Gradually yolk granules

begin to appear inside the cytoplasm and the egg grows in size. The yolk appears in scattered grains all round the germinal vesicle and is not concentrated at one pole as happens in the mysid *Mesopodopsis* (Nair, 1939). Consequently the germinal vesicle retains its central position as the egg enlarges. The increase in the size of the egg stretches the ovarian wall considerably until at last it appears as an extremely thin membrane forming the boundary line of the eggs which lie pressed against one another. The nuclei become attenuated. As the eggs are from the beginning lodged in the folds of the convoluted ovarian wall, most of the eggs in the sections of a ripe ovary appear as if they possess a membraneous covering. But in reality this membrane is only the thinned out ovarian wall. Due to mutual pressure the eggs assume polygonal shapes (Figs. 2 and 33).



FIGS. 1–2

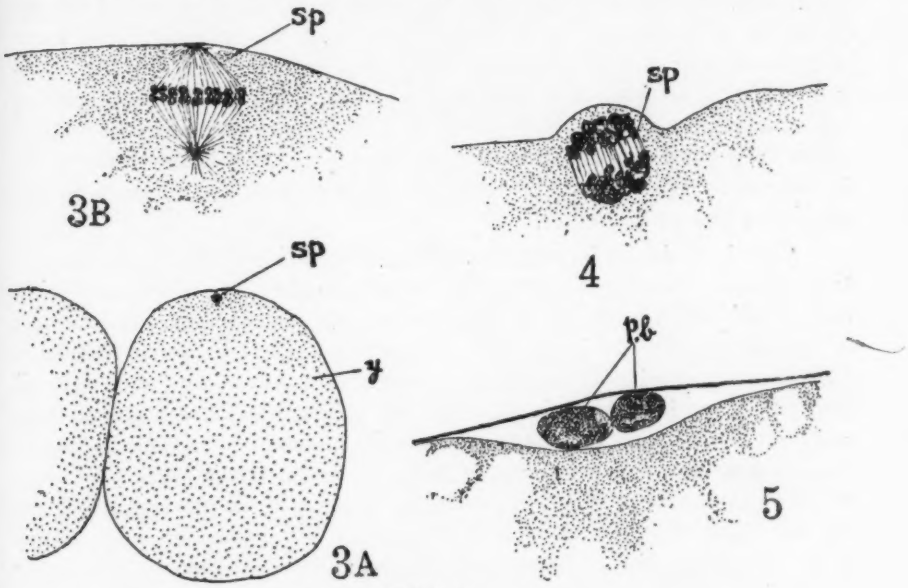
FIG. 1. *Squilla wood-masoni*. Section of the ovary showing the early oocyte. × 400.
FIG. 2. *S. wood-masoni*. Section showing the fully grown egg with the diffuse nucleus. × 200.

Long before the eggs attain their maximum size within the ovary the large germinal vesicle breaks down. This is interesting because, usually the breaking down of the germinal vesicle marks the termination of the growth period of the egg. In *Mesopodopsis* this happens just before the extrusion of the egg and the first polar spindle is formed immediately. But in both the species of *Squilla* investigated, the germinal vesicle breaks down much earlier. Moreover it does not mark the onset of the polar divisions. In the centre of the egg where the large germinal vesicle was situated appears a small stellate area of protoplasm with blue patches in it. This is the only indication of the presence of a nucleus in the egg at this stage. The staining effect suggestive of the presence of diffuse chromatin is obtained only when the slide is differentiated thoroughly. Otherwise the whole inner region of the stellate island of protoplasm goes dark blue and no structural details could be made out in it. In the final period of growth of the eggs inside the animal, in both the species, this is the only trace of the nucleus that could be found. There is no well-defined nuclear wall and the patches of chromatin are very diffuse. The shape is irregular, rather elongated than circular. All round the central island there is yolk and a peripheral layer of cytoplasm cannot be distinguished. Even in the ripest ovary sectioned this is the appearance that the eggs present.

4. The Extruded Egg

The eggs as they come out of the genital orifice are rounded in shape and show a tight fitting membrane. The egg of *S. raphidea* freshly extruded, measures 497 microns in diameter and that of *S. wood-masoni* 438 microns on an average. Large numbers of the eggs of both the species were fixed directly as they were coming out of the genital pores and also after intervals of one to five hours in succession, after which time the mother animals discarded the egg mass and it was spoilt. Sections of eggs of both the species fixed immediately they were extruded show the first polar division in progress. In between the eggs is found a thick substance. The nucleus by this time has abandoned its central position and migrated to the periphery and invariably in all the eggs of this lot the first polar spindle is clearly formed. The spindles are very small and lie at the very rim of the egg in a little star-shaped area of protoplasm (Figs. 3A and B and 34). Most of them are found to be in the early metaphase; but slightly older stages are not rare. They lie with their axes almost perpendicular to the circumference of the egg. It is worth noting that among the large number of eggs sectioned at this stage, not one egg could be found which was in a markedly earlier or later phase of division. This is the condition in both the species. The process of maturation and reduction therefore starts simultaneously and when the nuclei are in

the metaphase of the first polar division the eggs are extruded. The achromatic figure though small is of the amphiastral type and shows sharply



FIGS. 3–5

FIG. 3. (A) *S. raphidea*. Section of the egg as it is just extruded showing the first polar spindle. × 80. (B) Same. The spindle magnified. × 1200. FIG. 4. *S. raphidea*. First polar spindle: anaphase. × 1200. FIG. 5. *S. raphidea*. The polar bodies. × 1200.

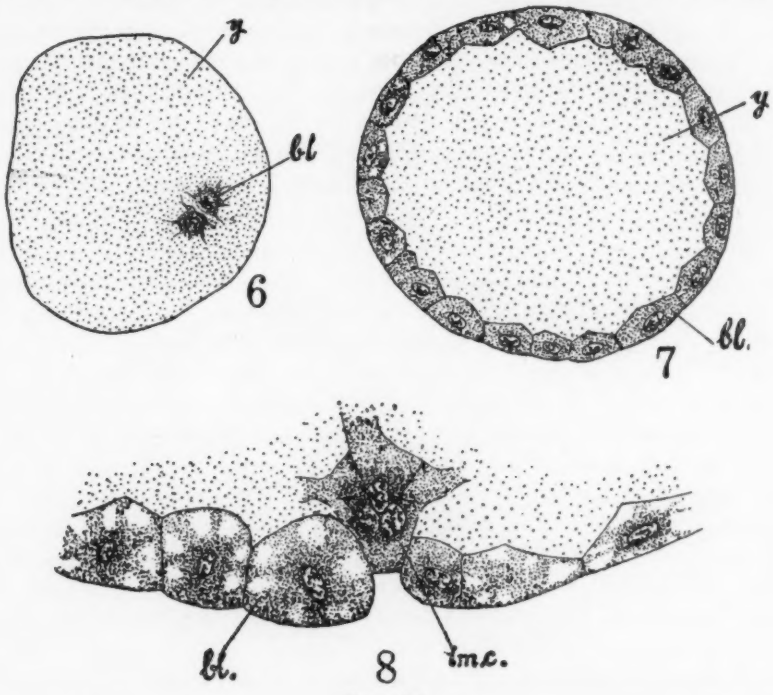
marked astral rays. The presence of central bodies and other details of the central apparatus could not be decided due to the extremely small size of the spindle. In eggs fixed about two hours later the division figures are seen to have reached the anaphase (Figs. 4 and 35). In some eggs the outer half of the nucleus lies outside the general margin of the egg in a small conical protuberance of protoplasm. This is the first polar body about to be rounded off. In about two hours' time, the first polar division is completed. The polar bodies are very minute and often difficult to distinguish. They appear as dark spots between the margin of the egg and the covering membrane (Fig. 5). After the first polar division the nucleus remains at the periphery and appears well defined and spherical. The second polar division takes place in exactly similar manner and it is completed in about four hours' time. In some of the eggs fixed at about this time two dark bodies could be seen lying side by side within the covering membrane (Fig. 5). Obviously these are the polar bodies. The next lot of eggs were the ones fixed more than six

hours after extrusion. In these many showed a single rounded nucleus situated in an island of protoplasm at the centre of the yolk. But it cannot be decided whether these are only the pronuclei which have sunk from the periphery or the zygote nuclei formed as a result of syngamy. The latter process was not observed; neither was it possible to identify the male pronucleus in the sections. The exact spot where the union of the two pronuclei takes place cannot, therefore, be ascertained. It is likely that, as in most forms where these processes have been studied, the female pronucleus sinks to the interior of the egg after the extrusion of the polocytes and unites with the sperm nucleus at the centre.

There is no doubt, however, regarding the situation of the zygote nucleus. It is internal. Among the eggs of *S. raphidea* fixed six hours after extrusion there is one which shows the first segmentation just completed. The products of this division lie deep in the yolk and very close to each other. They have only just separated and the nuclear membranes have not been formed (Fig. 6). Each blastomere is an irregularly shaped mass of protoplasm with radiating strands. In the centre of these are clumps of chromatin. In the next stage obtained the segmentation has proceeded one step further. This is also from *S. raphidea.* The cells are similar to those of the previous stage, but the nuclei are better reconstituted (Fig. 37). The nuclear membranes are clear and the islands of protoplasm are typically star shaped. They lie far removed from one another and one of the blastomeres is somewhat near the surface of the egg. No further stages in the segmentation of either form have been obtained. In the next set of eggs obtained the balstomeres have already risen to the surface of the egg. It is noteworthy, however, that in the first two divisions described above, no cleavage furrows are formed. The division of the zygote nucleus does not affect the egg as a whole. There is no indication of it either on the surface or in the inner yolk mass. The two stellate blastomeres merely move apart. In good preparations the yolk remains compact and unfragmented. In the second cleavage also the same thing happens. Thus, though it has not been possible to observe how the blastomeres finally rise to the surface, it may, I think, be reasonably inferred from the manner in which the first two segmentation divisions take place that in *Squilla* the cleavage is of the *Hemimysis* type (Manton, 1928).

Embryos of both the species showing all the blastomeres arranged evenly on the surface have been obtained. These correspond to the earliest stage described by Komai (1924), for *Squilla oratoria* and called by him the Blastula Stage. Komai counted from 200 to 300 cells on the surface of embryos in this stage. I find the number much smaller—particularly in *S. raphidea.* In this species the blastomeres appear very sparsely arranged on the surface and

in the earliest stage obtained the number is definitely below 100. The sparseness of the arrangement is quite clear in sections where the number of cells that could be counted along the circumference in any plane is never more,



FIGS. 6–8

FIG. 6. *S. raphidea.* Parasagittal section showing the first cleavage just completed. × 1800. FIG. 7. *S. raphidea.* Section showing the blastomeres at the surface. × 600. FIG. 8. *S. raphidea.* Section showing the beginning of gastrulation. × 1200.

and often less, than ten. All the same, they are arranged end to end and form a continuous belt enveloping the yolk. In the earliest embryos of this stage the blastomeres appear spindle-shaped in sections, the nuclei being situated in the middle. The cytoplasm is somewhat vacuolated and thins out in the internuclear regions. In surface view each blastomere is seen to be very expansive and polygonal in outline. In the younger embryos almost all the blastomeres are found to be in various stages of division. The cells multiply rapidly and along with this process an increase in their thickness also takes place. They lose their thin flat appearance and their inner boundaries get shifted more and more deeply into the yolk. In sections they are no longer spindle-shaped, but more or less polygonal (Fig. 7). The outer side

of each cell is still curved but the inner margin is triangular. Here also the cytoplasm is vacuolated, particularly near the inner margins. This gives it a stellate appearance in surface view. The nucleus is situated in an unvacuolated central region (Fig. 38). This appearance is presented by both the species in the late blastula stage, when the number of blastomeres becomes large. The mass of yolk enclosed by the ring of blastomeres remains homogeneous and but for the globulated structure characteristic of fatty material, shows no segmentation whatever. In most of the embryos at this stage, it could be noticed that the blastomeres of one side are larger and more closely arranged. Obviously this is the region of the germinal disc.

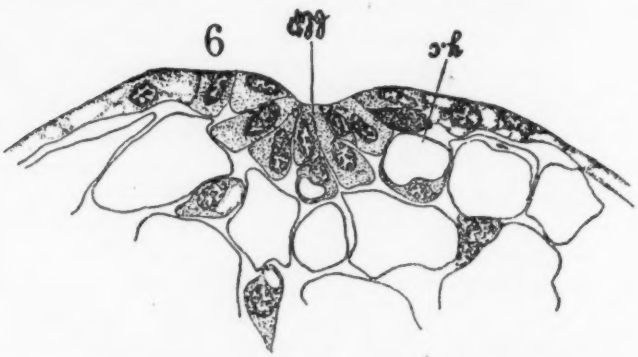


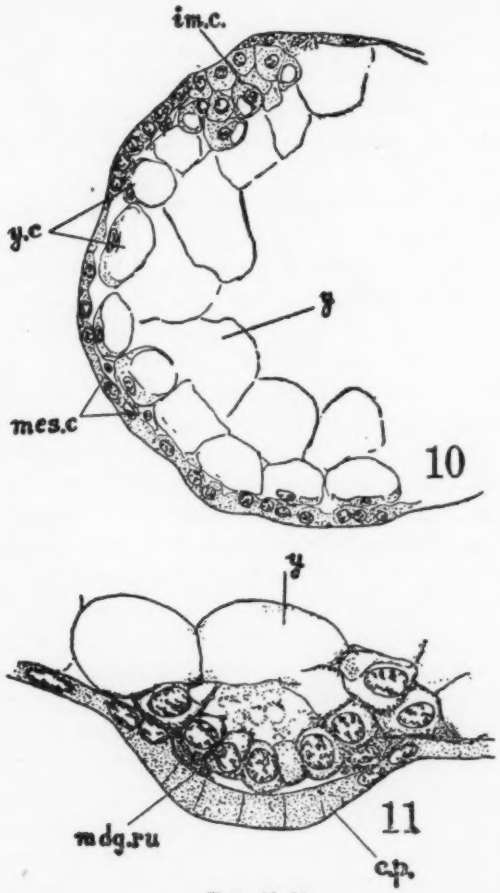
FIG. 9

S. raphidea. Section showing yolk-laden cells immigrating into the yolk from the blastoporal area. × 400.

5. *Gastrulation and the Establishment of Germ Layers*

Gastrulation takes place by immigration of cells from the centre of the large celled area referred to above. In surface view the cells of the germinal disc appear larger, but no differentiation into germ elements before the actual immigration takes place could be noticed. In the beginning the immigration is confined to one particular point which gets pitted. A few cells, usually only three or four, simply sink down. All the surrounding cells keep their position. This is clearly seen in Fig. 8, which is of a section of an embryo where gastrulation is just beginning. The cells that have sunk inwards lose their definite outline and become amœboid. They are starting to wander away from their point of origin. Their nuclei are small and the cytoplasm is drawn out into long strands. In both the species gastrulation commences in this manner. In the following stages more and more of these cells could be seen slipping in and wandering far into the yolk mass. As this process continues the gap which originally was very conspicuous is obliterated and

gradually the area surrounding the point is pushed in as a whole. This results in the formation of the familiar blastoporal depression characteristic of the Malacostraca (Fig. 9). Small cells continue to pass inwards from the area all the time this transformation of the original small pit into the large blastoporal depression is taking place. And by the time it is completed the germinal band assumes the characteristic V-shape. The blastoporal area comes to lie at the point where the two arms of the V meet. This stage approximately



FIGS. 10–11

FIG. 10. *S. raphidea.* Slightly parasagittal section of the germinal band showing the plug of cells below the blastoporal area, the layer of yolk cells below the germinal band, and the mesoderm cells of the naupliar region. × 200. FIG. 11. *S. wood-masoni.* T. S. through the base of the caudal papilla showing the mid-gut rudiment. × 600.

corresponds to stage IV of Komai (1924). Due to lack of material it has not been possible to make sufficient number of good whole mounts for a satisfactory surface study of these stages, and hence, no detailed account of the changes that take place in the germinal band can be given. The facts are almost entirely gathered from serial sections.

At the anterior end the two arms of the V are connected together by a bridge of cells. Aggregations of cells indicating the positions of the naupliar appendages also are formed but the order in which they make their appearance could not be determined. Komai found that the mandibles are the first to appear and that the antennæ and the antennules are formed a little later and simultaneously. In sections a row of large cells could be distinguished almost surrounding the blastoporal depression. These undoubtedly are the ectodermal teloblasts.

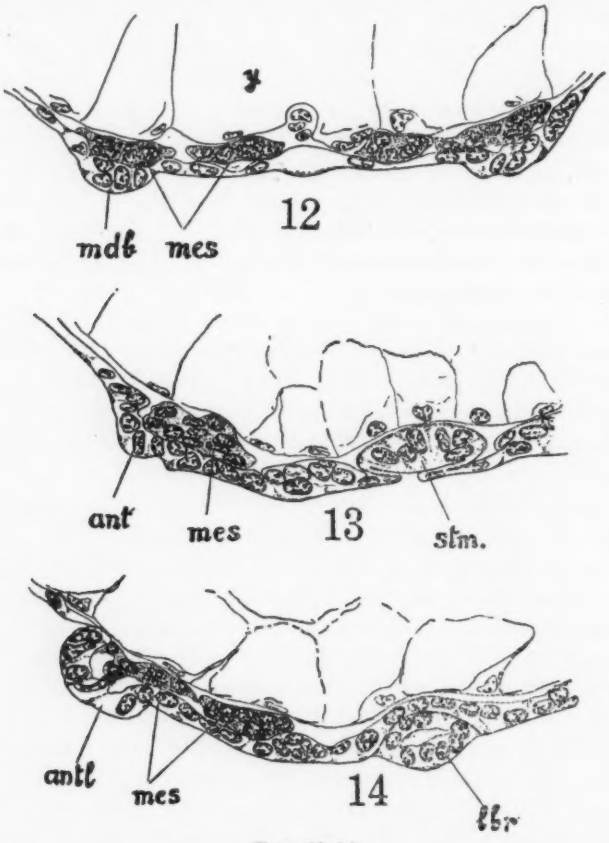
It has already been stated that the first cells that slip in at the blastoporal area lose their definitive boundaries and spread far into the yolk. But the later ones do not move away so much from the blastopore. The cells are all of one kind in the beginning, but very soon those lying in immediate contact with the yolk mass begin to absorb yolk and get distended with it. The same thing happens to the cells which slipped in first too. A good number of the later formed yolk-laden cells also wander through the yolk without any order, but many take a course along the surface of the yolk mass and assume positions below the cells of the germinal band. The other cells, *viz.*, those that do not become yolk laden, slowly move forwards through the interspace between the yolk cells and the ectoderm. They remain flat and unvacuolated and their cytoplasm is stained slightly deeper by hæmatoxylin. These are no doubt the mesoderm cells. It has to be noted that both these kinds of cells, *viz.*, the vitellophags which ingest yolk and the mesoderm cells which do not, arise from the same group of cells slipping in at the blastopore. The cells, as has been said already, show no differentiation whatever when they pass inwards from the surface. The differentiation takes place later. And no mesoderm cells are seen to be differentiated towards the posterior side of the blastopore. The blastoporal area is very large and the depression, from which cells continue to slip in, persists for a long time. But after the appearance of the naupliar appendages and the stomodæum the cells that pass in from the area invariably form yolk cells which remain crowded below it (Fig. 10). By this time the mesoderm cells that were originally differentiated spread forwards below the germinal band. They form the mesoderm of the naupliar segments. From the anterior extremities of the germinal band, in the region of the optic rudiments, a few slender cells slip inwards at this stage. These are the rudiments of the preantennulary mesoderm.

6. *The Caudal Papilla and the Mid-Gut Rudiment*

The next few stages obtained are exclusively from *S. wood-masoni*. No embryos of the other species corresponding to these stages have been obtained. In *S. wood-masoni* the next earliest stage obtained is not very much older than those described above. Here the caudal papilla has already been formed and the naupliar region has become well developed. Of the three pairs of appendages the antennules are the best formed and they show a slight bifurcation also. They stand raised from the body surface and appear as ectodermal tubes. The antennæ though also tubular are much shorter, while the mandibles are merely projections of the ectoderm. Rudiments of the maxillules and the maxillæ are visible, but are inconspicuous. The optic rudiments have become thick and more approximated with each other at the base. The stomodæum lies in a level with the antennæ and in front of it a prominent median projection marks the position of the labrum. The caudal papilla is small and somewhat oval in shape. The tip is bifid and reaches the level of the mandibles; standing a little raised from the body surface. It is broadest in the middle and the base is slender. The proctodæum could be seen as a narrow pit slightly behind the tip. It is on the morphologically dorsal side of the caudal papilla.

Internally the yolk cells have arranged themselves in a continuous row along the periphery of the yolk mass. Most of them have become very large by the ingestion of considerable quantities of yolk. The cytoplasm is thicker towards the outside where the nuclei are situated and extends as a thin rim round the yolk. In this way a major portion of the yolk mass of the embryo lying towards the periphery has become intracellular, but a central unabsorbed core remains. No yolk passes into the caudal papilla due to the presence of a block of large cells at its base. These are really the cells that slipped in last from the blastopore. It was said before that immigration continues for a fairly long period and that the last formed cells invariably become yolk-laden. The caudal furrow is formed in front of the blastoporal depression and as it deepens these cells are carried backwards. It is thus that they come to occupy a position at the base of the caudal papilla. Immediately after the formation of the caudal furrow immigration ceases and the proctodæum gets invaginated. The actual beginning of the proctodæal invagination has not been observed. In the earliest stage obtained, the invagination has already become fairly deep and its tip has come into contact with the block of cells at the base of the caudal papilla. But, I think the relative positions of these structures show that the proctodæum is formed exactly at the spot where the blastoporal depression was situated.

It is clear that the group of cells found at the base of the caudal papilla is the mid-gut primordium. At its edges it is already becoming epithelial and the yolk lying opposite to it has begun to break up (Fig. 11). The earlier formed heavily yolk-laden cells lying in contact with its edges are getting transformed into epithelial cells like those of the primordium. The nucleated cytoplasmic region of each yolk-cell becomes closely apposed to the edge of the primordium, its yolk vacuole bursts and the cytoplasm shrinks. A slight increase in the size of the nucleus also takes place simultaneoulsy. It will be shown later that this process continues till late in embryonic life and



FIGS. 12–14

FIG. 12. *S. wood-masoni*. T. S. of early embryo showing the mesoderm of the mandibular segment. × 400. FIG. 13. *S. wood-masoni*. T. S. of same stage showing the stomodæum and the antennal mesoderm of one side. × 400. FIG. 14. *S. wood-masoni*. T. S. of same stage showing the labrum and the antennulary mesoderm of one side. × 400.

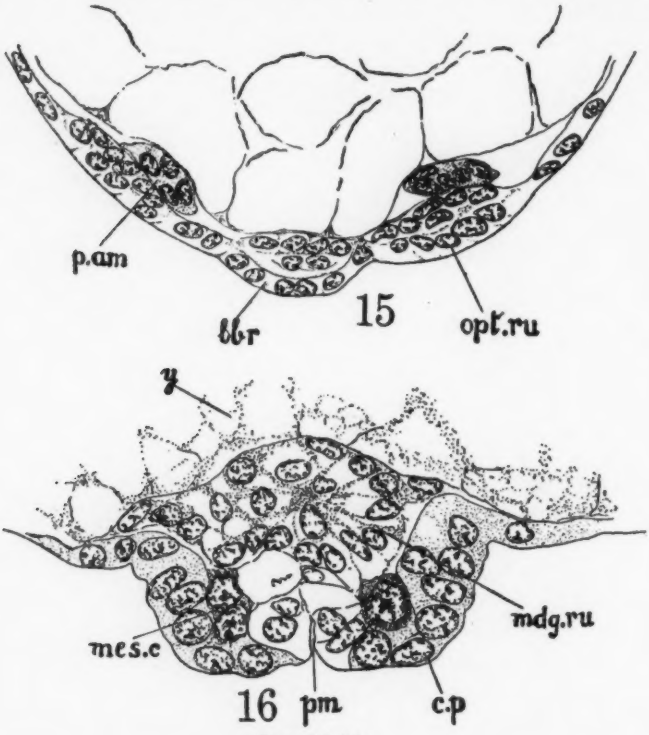
that thus it is that the mid-gut is formed (Fig. 40). No mitotic figures are seen in the cells of the primordium at this stage.

The cells of the proctodæum are characterised by the possession of very clear cytoplasm. They also possess large nuclei. The boundary between the general ectoderm and the cells of the invagination is clearly marked. In the space between the proctodæum and the mid-gut primordium on the one hand and the ectoderm on the other, lie the mesoderm cells. They are large and possess conspicuous nuclei. In transverse sections four such cells could be seen on either side forming a band. The two bands are not continuous and leave a space above the proctodæum. The dorsal-most cell of each group is larger than the rest. The ectodermal cells lying outside these are also large showing thereby that it is the region of the teloblasts (Fig. 16).

In the anterior region of the embryo the mesoderm bands of the naupliar segments have become segregated. The mandibular segment shows two blocks on either side of the mid-ventral line (Fig. 12). The outer of these lies at the base of the mandibular bud. There is as yet no differentiation into muscles. The row of yolk cells passes over these mesoderm blocks uninterruptedly. The antennæ are situated at a greater distance from the mid-ventral line than the mandibles and their mesoderm blocks are more massive (Fig. 13). The stomodæum which lies in this segment appears as a shallow invagination, dorso-ventrally compressed. Its dorsal wall is very thick. The antennules lie immediately in front and a little farther out. Their mesoderm blocks are larger still and show slight differentiation into muscles in the cavity of the appendage (Fig. 14). The labrum is well developed and thick walled. It shows no mesoderm cells inside. In front of this region, but more towards the mid-ventral line, lie the preantennulary mesoderm bands (Fig. 15). They are situated between the posterior region of the optic lobes and the layer of yolk cells. The bands are rather small and do not extend far. The optic lobes have become thick and the cells have got themselves arranged in layers.

The next stage obtained is also of *S. wood-masoni*. Fig. 17 shows a median sagittal section of this. It corresponds approximately to Stage IX of Komai. Only the first seven pairs of appendages are present. The caudal papilla has become considerably elongated. The antennules and the antennæ are long and tubular and the former shows distinct bifurcation. They lie parallel to each other and are directed sideways and backwards. The mandibles, maxillules and maxillæ appear only as projections of the body surface. They are not tubular. The first and second pair of thoracic appendages on the other hand are well developed and are fairly long tubular processes. The succeeding thoracic segments though clearly formed do not possess appendages.

The apex of the caudal furrow lies against the third thoracic segment. The caudal papilla is dorsoventrally compressed and lies closely apposed to the



FIGS. 15–16

FIG. 15. *S. wood-masoni.* T. S. of the same stage as Fig. 14 showing the optic rudiments and the preantennulary mesoderm bands. × 400. FIG. 16. *S. wood-masoni.* T. S. of the caudal papilla showing the proctodæum and the mid-gut rudiment. The mesodermal teloblasts are also seen. × 600.

main body of the embryo. Its tip is blunt and shows no marks of even a vestigial furca (Fig. 19). Segmentation is not clear in the distal part of the caudal papilla. The ectodermal and mesodermal teloblasts are seen undergoing active division a little distance from the tip (Fig. 39). Their descendants lie in irregular rows behind. Transverse sections of this stage show that a mid-ventral ectodermal teloblast is present (Fig. 18).

The mesoderm of one of the posterior thoracic segments in this stage is shown in Fig. 20. It consists of two blocks of cells with large nuclei, lying on either side of the mid-gut. No differentiation is as yet visible in these cells; but anteriorly near the angle of the caudal furrow, rudiments of the

ventral longitudinal muscles could be seen on the inner side of the morphologically lower half of each mesoderm block. Intersegmental connections are also being established between these along either side. Each block extends upwards to a little distance from the mid-dorsal line. From this point

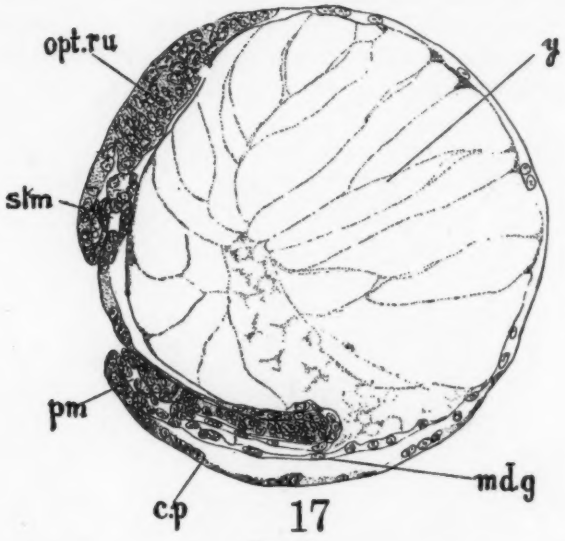


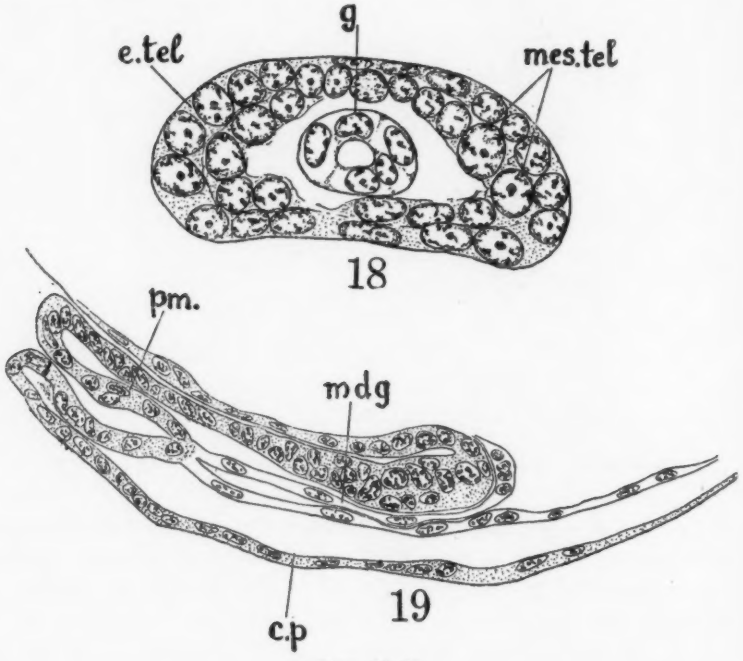
FIG. 17

S. wood-masoni. Sagittal section of the entire embryo of the stage next to that shown in Figs. 11–16. × 200.

(in the anterior thoracic segments) a tongue of mesoderm cells grows down and gets attached to the outside of the mid-gut. These processes which are only just beginning in this stage are clearly seen in the next and will be more fully described thereunder.

The anus is shifted from the dorsal position of the previous stage to a terminal one (Fig. 19). It lies in the cleft between the two terminal lobes of the caudal papilla. The proctodæum is thick walled and has not elongated much. It extends roughly as far as the junction betweeen the thorax and the abdomen. The mid-gut on the other hand is very long. It is somewhat funnel-shaped, the tubular portion inside the caudal papilla representing the tail. The mouth of the funnel is placed at the junction between the body of the embryo and the caudal papilla. Its front edge is bent forwards over the cell layers of the anterior thoracic segments and the hinder edge extends upwards for some distance along the periphery of the yolk mass. It is noteworthy that the cells of the mid-gut rudiment do not show any division figures

at all. That part of it which lies in the caudal papilla shows very few nuclei and the cells are thin and long drawn out. This. no doubt, has been brought about by the rapid growth of the caudal papilla and the shortness of the proctodæum. The shifting of the anus from the dorsal to the terminal position may also exert a pull on the mid-gut rudiment. There is no intercommunication between the cavities of the proctodæum and of the mid-gut tube. Small quantities of disintegrating yolk pass into the latter from the hinder part of the yolk mass.



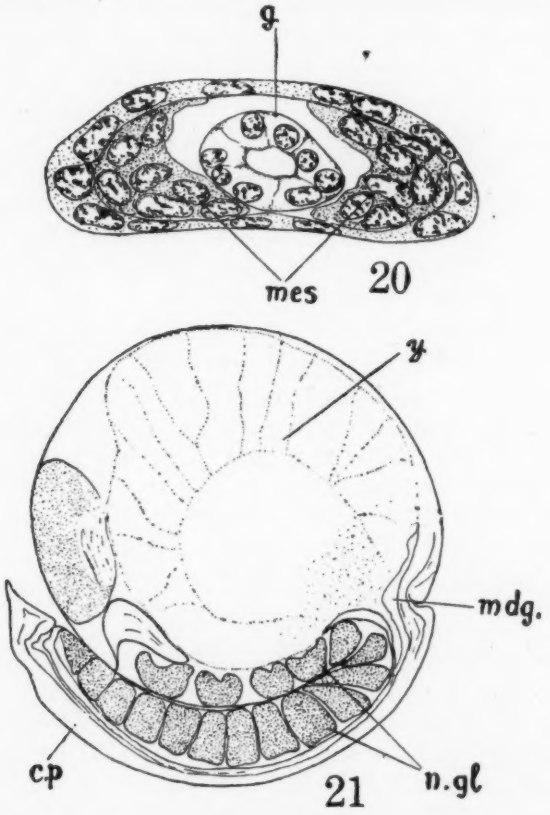
FIGS. 18–19

FIG. 18. *S. wood-masoni.* T. S. of the caudal papilla showing the descendants of the ectodermal and mesodermal teloblasts. Same stage shown in Fig. 17. × 600. FIG. 19. *S. wood-masoni.* Same stage. Sagittal section of the caudal papilla showing the proctodæum and the mid-gut. × 400.

The yolk cells reach their maximum growth at this stage. It is clearly seen in the median sagittal section shown in Fig. 17. The transformation of these cells into the mid-gut epithelium has not progressed far into the yolk mass and hence only a small quantity of yolk has broken down. Most of it has been ingested by the yolk cells, which enlarge considerably and arrange themselves radially. Their nuclei are situated towards the periphery. There

is a narrow, but continuous space between the outer sides of these enlarged yolk cells and the general ectoderm of the surface.

In the head region a fold of ectoderm has grown in between the thick optic lobes and the layer of yolk cells. It begins from the top and gradually grows downwards. The preantennulary mesoderm bands have by this time elongated considerably. At the upper end they become closely apposed to the ingrowing ectodermal fold and at the lower to the stomodæum. A block of cells from these bands have descended into the cavity of the labrum and are forming muscles. Behind the antennulary ganglion the cells of the preantennulary mesoderm are fast getting transformed into muscle strands.



FIGS. 20–21

FIG. 20. *S. wood-masoni*. T. S. of the caudal papilla showing the mesoderm of a typical trunk segment. Posterior thoracic region. × 600. FIG. 21. *S. raphidea*. Sagittal section of the entire embryo of the latest stage obtained showing the ganglia. × 200.

The mesoderm of the antennulary and antennal segments is obscured by the enormous growth of the nerve ganglia, but most of it has descended into the cavity of the appendages to form muscles. The stomodæum at this stage is directed upwards and forwards and its tip is swollen (Fig. 17). The mesoderm of the mandibular segment has become more massive than in the preceding stage and lies at the base of the appendage. In this as well as in the succeeding segment there is an intucking of the ectoderm from either side showing the formation of the endoskeleton. Rudiments of the adductor muscles are also seen. In the maxillary segment the mesoderm is fragmentary. It is better developed in the first two thoracic segments where considerable differentiation into muscles has taken place. Some of these strands pass upwards along the sides of the mid-gut rudiment and get attached to the dorsal ectoderm. The carapace is being formed by an intucking of the ectoderm of the dorsal side above the point of origin of the caudal papilla.

A few more broods of embryos of both the species, all older than the stage just now described, were obtained. These differ only very slightly in age and so may be described under one head. They correspond approximately to Stage X of Komai. The abdomen shows only four pairs of appendages. There is faint pigmentation in the optic lobes. In the living condition the mid-gut rudiment pulsates actively. The second thoracic appendage has grown very large and broad. Its tip reaches as far as the base of the antenna. The caudal papilla is very long.

The ventral nerve cord has become thick and division into ganglia is clear. Behind the stomodæum, 16 ganglia are marked out (Fig. 21). Of these the first is the mandibular, then there are the maxillulary and the maxillary. The eight ganglia behind these belong to the thoracic segments. The caudal papilla is flexed over exactly opposite the third thoracic ganglion. In the abdomen though the fifth ganglion is distinctly marked out there is no corresponding external segmentation. Behind this ganglion there is a small tapering mass of nerve cells representing the sixth ganglion.

The anus is situated a little distance behind the tip of the caudal papilla on the ventral side. As a result of this shifting of the anus from a terminal to a ventral position the proctodæum is bent downwards and lies for a short distance perpendicular to the axis of the caudal papilla. From the angle so formed the proctodæum gives rise to two pouches on either side. These are evidently the rudiments of the rectal glands (Fig. 30). The mid-gut inside the caudal papilla has become considerably drawn out and its lumen appears very narrow. Four or five cells comprise the entire circumference of the tube. The cytoplasm of the cells is clear. Free communication has not yet been

established between the mid-gut and the proctodæum. The junction between the two lies in the anterior abdominal segments. The front end of the mid-gut rudiments is growing rapidly by the transformation of the yolk cells. Division figures begin to appear at many points in this. It extends far on either side. The lateral extensions are beginning to curl inwards leaving a

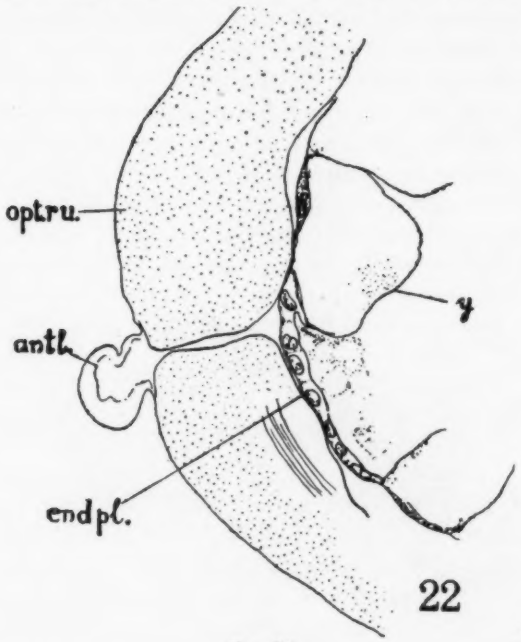


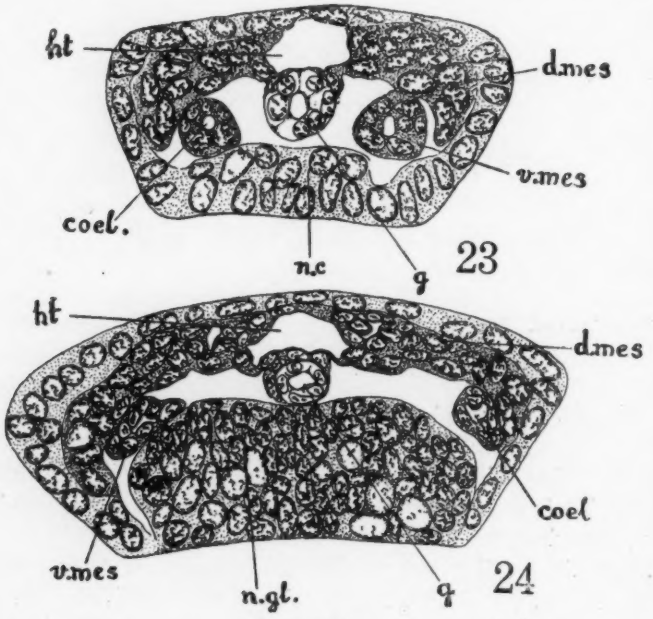
FIG. 22

S. wood-masoni. Sagittal section of the anterior end showing the formation of the endoderm plate opposite the optic rudiments. × 400.

central groove (Fig. 28). Their cells are larger than those of the median groove. In the oldest embryos studied these lateral folds of the endoderm plate have become completely curled off, their edges meeting to form a tubular structure on either side of the mid-gut (Figs. 31–32). These are the primary liver tubes. Their posterior ends are blind, while the anterior edges continue to grow forwards by the addition of modified yolk cells. The lower edge of the mid-gut tube that lies in the middle is continuous with the ventral row of yolk cells. The lumen of all the three tubes contains disintegrated yolk. The yolk cells on the dorsal side remain in tact. At the centre, the area of disintegrating yolk has increased. In the older embryos a further progress is made in the transformation of the yolk cells. At two points opposite the boundary between the brain and the optic lobes two small endoderm plates

appear independently of the hinder funnel-shaped mid-gut rudiment and the liver lobes (Fig. 22). These two areas are unconnected and lie some distance in front of the stomodæum. Disintegration of yolk takes place against their inner surface. These are probably the rudiments of the anterior liver tubes.

Between the optic lobes a thickening of the ectoderm takes place in front. This marks the position of the rostrum. The ectodermal folds behind the lobes have grown deeper down. Similar folds have also grown in from between the sides and also from between the optic lobes and the brain. The stomodæum is considerably enlarged. The upper end is swollen and thick walled and is directed backwards. The tip lies in the maxillulary segment. The labrum is somewhat dorsoventrally compressed. Groups of ectodermal cells on its ventral wall have enlarged considerably and show deeply staining granules in their cytoplasm. Their nuclei also are big. Evidently these cells are becoming glandular in nature. From the front wall of the stomodæum a diverticulum has been formed, and this grows upwards through the



FIGS. 23–24

FIG. 23. *S. raphidea*. T. S. of the caudal papilla showing the early differentiation of the trunk mesoderm. × 600. FIG. 24. *S. raphidea*. T. S. of the trunk segment. Later stage than shown in Fig. 23. × 750.

cavity of the labrum (Fig. 27). Its blind end comes to lie in a level with the upper edge of the stomodæum over which the layer of yolk cells passes in unbroken array.

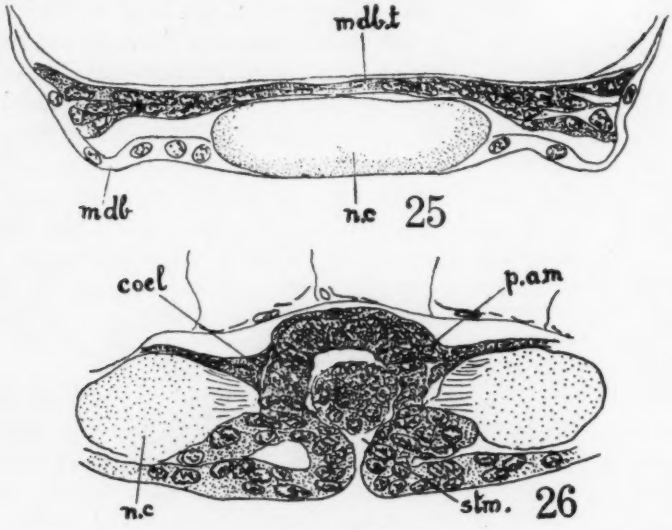
7. *The Mesoderm*

The lower ends of the preantennulary mesoderm bands which were seen to get closely apposed to the front side of the stomodæum in the preceding stage have now grown round it so as to form a thin envelope (Figs. 26–29). Its cells are flat and the nuclei slender and a narrow cœlomic space develops in it. This extends almost all round the stomodæum. Only for a short distance in front of the stomodæum do the strands lie closely apposed to each other. Beyond that they diverge and run upwards close behind the hinder surface of the ectodermal fold. Large number of muscle cells have been formed in them. There is no indication of the dorsal organ. In the mandibular and the maxillulary segments the adductor muscles are very well developed.

8. *The Mesoderm of a Typical Trunk Segment*

The growth of the mesoderm of the maxillary and the first three thoracic segments has not been followed in any great detail. The following description applies only to the segments which lie behind this limit. It was said while describing the preceding stage that the mesoderm of each of these segments gets divided into two blocks occupying a lateral position and closely adhering to the inner surface of the body wall. In the beginning the lower end of each lateral block extends as far as the mid-ventral line below the mid-gut or the proctodæum as the case may be. But the growth of the ventral nerve cord pushes them apart. The nerve cord becomes very thick and occupies almost all the available space below the gut. The mesoderm block on either side gets divided into a dorsal and a ventral part. The ventral part appears rounded in cross section. Very soon a cœlomic space appears in this ventral mesoderm and the cells around it begin to transform themselves into muscle rudiments (Fig 23). They show elongated nuclei and as they get modified in this manner the ventral mesoderm of successive segments gets connected together to form a continuous band on either side. No intercommunication between the cœlomic spaces of adjacent segments takes place. This is the beginning of the ventral longitudinal muscle. These lateral muscle strands extend from the maxillary to the last abdominal segment. The dorsal mesoderm block has by this time extended a tongue of cells to the gut wall, thereby enclosing a space on the dorsal side. This is the heart cavity. At first it is bounded on the top by the body wall, below by the gut and on either sides only by the mesoderm. But the mesodermal tongues that extend downwards

to the gut wall soon spread round it forming an investment of cells all round. This makes the floor of the heart cavity mesodermal. Later the edges of the mesoderm blocks at the top advance towards each other and meet in the centre (Fig. 24). Thus the heart cavity comes to be bounded on all sides by

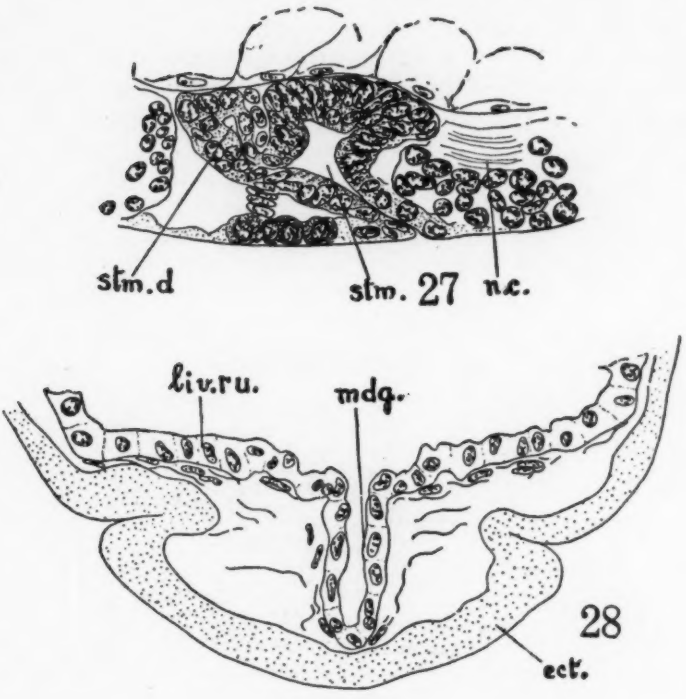


FIGS. 25–26

FIG. 25. *S. raphidea.* T. S. of the head region showing the mandibular tendon. × 600.
FIG. 26. *S. raphidea.* T. S. of the stomodæal region showing the preantennulary mesoderm and the cœlomic spaces in it. Same stage as Fig. 21. × 600.

mesoderm. When such a condition is attained the cavity appears roughly crescentic in cross section. By this time an irregular cœlomic space appears in the dorsal part of the mesoderm blocks. They lie on either side of the heart. In the oldest embryos sectioned the outer walls of these cœlomic cavities are seen to be getting transformed into muscles. The lower end of the dorsal mesoderm of each segment grows downwards between the body wall and the ventral mesoderm. The pericardial floor bases and the limb mesoderm are formed from this region.

The mesoderm of the last abdominal segment is very fragmentary and no cœlomic space is developed in it. It lies as a group of cells on either side of the proctodæum where the latter bends ventrally. Laterally it gets connected to the ventral longitudinal muscle rudiments of the preceding segment. A few cells also wander upwards, and at the dorso-lateral angles, bridge the space between the gut and the body wall, thereby enclosing a



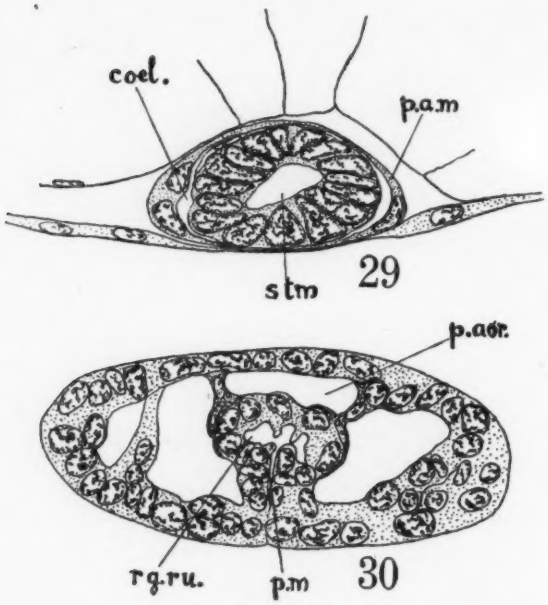
FIGS. 27-28

FIG. 27. *S. raphidea.* Sagittal section of the stomodæum showing the diverticulum. × 600.
FIG. 28. *S. raphidea.* Same stage as Fig. 21, showing the mid-gut epithelium. × 400.

cavity similar to that of the heart in the anterior segments. At the lower end, these cells spread over and invest the rudiments of the rectal glands (Fig. 30).

9. *Endoskeleton*

In the mandibular and maxillulary segments the ectodermal intuckings from either side, mentioned under the preceding stage, grow inwards and fuse in the centre forming the tendons of the adductor muscles (Fig. 25). They are large and thickly nucleated especially at the sides. Similar intuckings though smaller in size occur in all the succeeding segments also. Vertical intersegmental bars grow upwards from the mid-ventral line through the nerve cord and get connected to the horizontal bars. These are best seen in the mandibular and maxillulary segments.



FIGS. 29–30

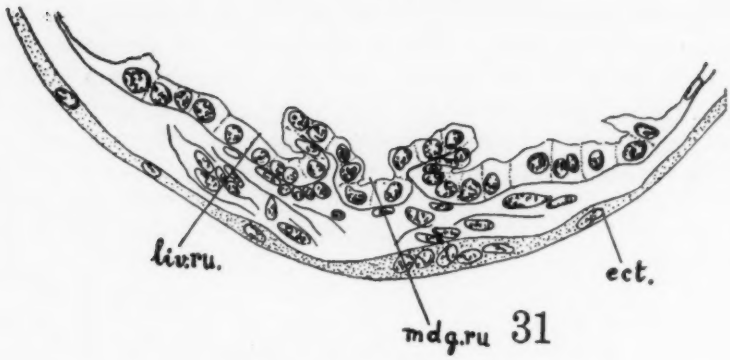
FIG. 29. *S. raphidea.* Same stage as shown in Fig. 24. T. S. of the stomodæum showing the cœlomic spaces in the preantennulary mesoderm. × 1200. FIG. 30. *S. raphidea.* Same stage as shown in Fig. 29. T. S. of the caudal papilla showing the formation of the proctodæal diverticula and the posterior aorta. × 600.

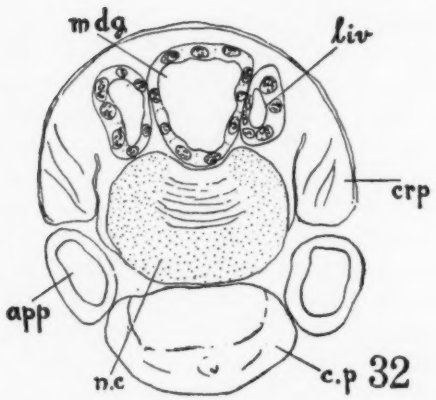
The carapace is well developed and stands a little raised from the body on either side. Mesoderm cells from the maxillary and the first thoracic segments grow upwards on either side and form the musculature of the carapace.

No indication of the maxillary gland was seen even in the oldest embryos obtained.

10. Discussion

The growth of the egg within the ovary of *S. raphidea* and *S. wood-masoni* takes place in a manner that is commonest in the Crustacea. The fully grown egg is typically centrolecithal. The only peculiarity that has been observed is in the breaking down of the germinal vesicle. The nuclear wall disappears much earlier than is usual, when the nucleus is still at the centre of the egg and deposition of yolk is not complete. The polar spindle is not formed immediately and the nucleus waits in this indistinct condition for some time. Many cases are known among the Arthropoda where towards the end of the growth period of the egg the nucleus totally disappears from view. Such a





FIGS. 31–32

FIG. 31. *S. wood-masoni.* Formation of the liver tubes from the mid-gut epithelium. T. S. × 400. FIG. 32. *S. wood-masoni.* T. S. of the liver and the mid-gut tubes. × 200.

thing happens, for instance, to take a recent example, in *Hanseniella* (Tiegs, 1940). In *Squilla* the nucleus does not totally disappear, but remains in an ill-organized condition. The meiotic processes are interesting in the extremely small size of the polar spindle and also in the fact that the spindles are of the amphiastral type. In this latter feature *Squilla* shows a marked difference from the mysid *Mesopodopsis*, although there is resemblance between the two forms in the position and somewhat also in the size of the achromatic figure.

The zygote nucleus is central in position and cleavage up to the four-celled stage, further than which it was not observed, takes place exactly as in the mysids, *Hemimysis* (Manton, 1928) and *Mesopodopsis* (Nair, 1939) and also in *Nebalia* (Manton, 1934). No cleavage furrows that give rise to

the so-called primary yolk pyramids in most of the Decapoda, are formed. These two types of cleavage, Manton has pointed out, are totally different and cannot accurately be included under one title as the " Superficielle Furchung " of Korschelt and Heider. Thus we find that in the mode of cleavage *Squilla* resembles the Peracarida and Leptostraca and differs from the Decapoda. In this connection it has to be pointed out, however, that Zehnder (1934) in his very detailed study of the early embryology of *Astacus* has not shown the cleavage furrows on which Sollaud (1923) has laid stress previously. Possibly this is an exception and the type of cleavage described by Sollaud may be considered typical for the Decapoda. *Squilla* differs in a much greater degree in the matter of cleavage from the Syncarida in which cleavage has now been shown to be holoblastic (Hickman, 1937).

Unlike the Peracarida no superficial differentiation of germ layers takes place in *Squilla*. A blastoporal depression is formed and from there cells of one kind migrate inwards. Most of these cells ingest yolk and wander into the yolk mass, while a few lying near the anterior edge of the balstoporal depression form the mesoderm of the naupliar segments. Thus the cells that pass inwards from the blastopore are mesendodermal in character. This process is quite similar to the gastrulation in the Palæmoninæ (Sollaud, 1923) and in *Nebalia* (Manton, 1934), though the accumulation of mesendodermal cells below the blastopore to form the " bouchon blastopoique " is not so very conspicuous. This late differentiation of the germinal elements shows that the early development of *Squilla* is less determinate than that of the Peracarida as exemplified by *Hemimysis* and *Mesopodopsis*. The mesoderm is differentiated from the anterior mesendodermal cells and this has been shown by Manton (1928) to be one of the chief characteristics of the Malacostraca. It may be noted here that the rising of the blastomeres to the surface of the yolk, and the subsequent immigration of cells into the interior from the blastoporal depression, if viewed from the angle of the ' multiphased gastrulation theory ' of Roonwal (1939), can be considered as two phases of a temporally elongated process of gastrulation. Roonwal distinguishes three types in the second phase among the Crustacea and *Squilla*, according to this scheme, comes under ' intermediate type ' where gastrulation ceases simultaneously with the formation of the caudal papilla.

The changes in the external form of the embryo are of the Decapodan and Leptostracan type. So also are the arrangement and further growth of the ectodermal and mesodermal teloblasts. It has unfortunately not been possible to determine the mode of origin and the exact number of the teloblasts and a comparison with other forms regarding this aspect cannot be made. The presence of a midventral ectodermal teloblast is a point of

resemblance with the Peracarida. The teloblasts, however, occur in a complete ring round the blastoporal area, and by division, form the walls of the caudal papilla on all sides. This is probably due, as has been suggested, (Manton, 1934), to the fact that no yolk enters the caudal papilla. The teloblasts form all the post-naupliar segments of the body and in this *Squilla* differs from the Decapoda where, according to Sollaud (1923), they form only the post-maxillary segments.

The mid-gut primordium is formed from the group of cells that slip in last from the blastopore. It was said that only one kind of cells migrates inwards during gastrulation and that these are mesendodermal in nature. Hence, strictly speaking, the mid-gut primordium has to be considered mesendodermal in origin. But these cells pass in only late and by the time, differentiation of the head mesoderm cells is over and the teloblasts have been formed. It may, therefore, be reasonable to consider the mid-gut rudiment as practically endodermal. Such a view is possible because the cells of the primordium slip in at a later time than in the Decapoda and the Leptostraca. In these two groups the cells that comprise the rudiment are formed early and move backwards from under the blastopore. There they form a regular plate—the " lame intestinale primitive " of Sollaud (1923) and the epithelial endodermal plate of Manton (1934). Later, yolk cells lying close to the edges of this plate get transformed and are added on to it and the plate grows to form the epithelium of the mid-gut. In *Squilla* the process is essentially the same, the only difference being the slightly later formation of the cells of the rudiment and their immediate growth in extent by the addition of modified yolk cells. These yolk cells and the cells of the primordium, it has to be remembered, belong to the same category. The former differentiate earlier from the mesendodermal mass by ingesting yolk, and wander into the yolk mass, finally getting themselves arranged along its periphery. The cells of the rudiment on the other hand are the last to slip in from the blastopore. Some of these cells lying close to the yolk mass ingest small globules of yolk also. Thus we see that the entire mid-gut is formed from cells which are all of one kind. These may be considered either endodermal because they ingest yolk, or mesendodermal in view of the absence of superficial differentiation of the germ layers and the common origin of the mesoderm and the endoderm. In *Leander* the early formation of the primordium led Sollaud to the view that it is mesodermal in origin. This has been proved to be unjustified (Manton, 1934). In the development of the mid-gut, therefore, *Squilla* resembles the Decapoda and *Nebalia*. It may be pointed out in this connection that the appearance presented by the mid-gut and the proctodæum in *Squilla* is strikingly similar to the figures given by Sollaud for the

Palæmononæ. Moreover the mode of origin of the mid-gut in *Squilla* seems to show that the difference exhibited in this respect by the Decapoda and *Nebalia* on the one hand, and the Peracarida on the other, is only one of degree and not of kind. In the latter group all the endoderm cells ingest yolk and get arranged round the periphery. Transformation of this layer of yolk cells into the mid-gut epithelium starts later opposite the tip of the proctodæum and the stomodæum. In *Squilla* the proctodæum is invaginated from the blastoporal area and the cells that slipped in form the latter before this happens come to lie against the tip of the invagination. These cells constitute the mid-gut primordium and some of them, it was shown, also ingest yolk. They can therefore be considered as yolk cells forming a continuous row with the earlier formed ones arranged along the periphery and their further growth is exactly similar to that of the posterior endoderm plate of *Hemimysis* and *Mesopodopsis*. Thus in *Squilla* the posterior endoderm plate is formed much earlier than in the Peracarida. In the Decapoda and *Nebalia* it is formed earlier still. This difference is probably due to the precocious appearance of the proctodæum in these forms.

The liver is formed as diverticula from the mid-gut epithelium on either side and the process is similar to that of *Nebalia*. The significance of the two epithelial modifications of the yolk cells found at the anterior region cannot be ascertained since no further stages were obtained so as to follow their growth.

The initial lay-out and the early growth of the trunk mesoderm in *Squilla* are essentially similar to the same processes in *Nebalia* and differ markedly from those of the Peracarida. But the formation of a cœlomic space in the ventral mesoderm is a feature not found in any of the other groups. There is also another difference between the development of the somites in *Squilla* and *Nebalia*. In the latter the mesoderm block divides into two only after forming the floor of the dorsal blood vessel and also the gut musculature. But in the Stomatopod the two processes take place almost simultaneously. In the formation of the heart cavity, the gut musculature, the dorsal longitudinal muscle and the walls of the dorsal blood vessel *Squilla* very closely resembles *Nebalia*, up to the latest stage obtained. Cœlomic spaces also develop in the dorsal mesoderm, but this happens much later than in the ventral blocks. It is worth noting that these similarities in almost every detail of the development of the trunk mesoderm are, to some extent at any rate, due to the absence of yolk in the caudal papilla in both the forms.

There is nothing peculiar regarding the development of the preantennulary mesoderm. There is no yolk septum in *Squilla*, and the disposition of

these mesoderm bands is the same as we get in *Nebalia*. Even in the latest stage obtained there is no trace of a dorsal organ. The upper ends of the bands lie some distance apart, closely apposed to the fold of ectoderm behind the optic lobes. Whether the dorsal organ is yet to be developed it is impossible to say, but it has to be pointed out that Komai (1924) who examined a series of stages up to the one ready to hatch out, definitely says that it is absent.

11. Conclusion

The Stomatopoda forms an aberrant group among the Crustacea and their affinities have always remained the subject of discussion. That they belong to the Malacostraca has, I think, never been doubted, but opinion has differed very widely as to which order of that sub-class they are most closely related. A historical survey of the views held by different authors has been given recently by Balss (1938) and so need not be repeated here. Suffice it to say that this review and a consideration of the morphological characters of the group lead Balss to the opinion that the Stomatopoda are related to the Leptostraca and Anaspidacea on the one hand and to the Decapoda on the other. He considers the similarities shown by the Stomatopoda and Peracarida as without any phylogenetic significance. He illustrates these relationships by a phylogenetic tree with two trunks springing from the primitive Malacostraca, one leading to the Eucarida and the other to the Peracarida. On the Eucarida-trunk he shows the Stomatopoda-branch, springing mid-way between the Decapoda and the Anaspidacea, at the same level as the Leptostraca-branch on the other trunk. From his study of the course of external development of *Squilla oratoria* Komai (1924) arrived at practically the same conclusions as Balss and stressed the greater similarities with *Nebalia*. It will be seen from the foregoing discussion that embryological evidence emerging from the present investigation is strongly in support of this view. The development of the two species of *Squilla* as far as it has been followed shows closer similarities to that of *Nebalia* than of any other form. The only noteworthy differences are that in *Squilla* a dorsal organ is not developed and that the seventh abdominal segment of *Nebalia* is not represented even in vestigial form. With regard to the first point the finding of Komai is in agreement with that of the present author. But regarding the second one it is not known whether a seventh segment is developed and suppressed before the last stage where alone it has been possible to see the segmentation of the abdomen clearly. Nevertheless, the small size of the ganglion and the mesodermal somites of the sixth abdominal segment would appear to suggest that this is not probable. In spite of these differences it has to be admitted that the two groups are closely related because the resemblances in the other details of

developments are so great. And now that it is known that the development of *Anaspides* (Hickman, 1937) closely resembles that of *Nebalia*, the aforesaid view of Balss that the Stomatopoda are near relatives of the Leptostraca and the Syncarida gains support. But the occurrence of holoblastic segmentation in *Anaspides* is irreconcilable with this view.

The resemblances in development shown by *Squilla* to the Decapoda are striking. The mode of segmentation differs markedly from that described by Sollaud for *Leander* and other Palæmoninæ, but it is similar to that in *Astacus* as shown by Zehnder (1934) and years ago by Morin (see MacBride's *Text-book of Embryology*). The absence of the dorsal organ and the teloblastic origin of all the post-naupliar segments are no doubt important differences. But there is much similarity in most of the other details of development, such as gastrulation, formation of the mid-gut rudiment, the arrangement and growth of the teloblasts, the absence of a seventh abdominal segment and the changes in the external form of the embryo. On the other hand more differences than similarities are found when a comparison is made with the Peracarida. There is agreement in the mode of cleavage, the presence of preantennulary mesoderm bands and of a mid-ventral ectodermal teloblast, but on the whole the early development of the Stomatopod is less determinate than that of the Peracarida, especially the mysids. There is no superficial differentiation of the germ layers in *Squilla*. The disposition and growth of the teloblasts are totally different. The liver in the Peracarida is mesodermal in origin, but in *Squilla* it is endodermal. Similarly the growth and differentiation of the trunk mesoderm in *Squilla* take place in a manner not comparable to that of the Peracarida.

12. Summary

1. The early development of *S. raphidea* and *S. wood-masoni* of the Madras Coast has been worked out.

2. The growth of the egg inside the mother has been traced. The earliest oocyte shows a large central nucleus containing a prominent nucleolus.

3. The breaking down of the germinal vesicle takes place much earlier than is usual in most of the Crustacea.

4. When extruded the eggs are found to be undergoing the first polar division.

5. The spindle is extremely small and is situated at the periphery of the egg.

6. The mode of cleavage is the same as in the Peracarida and the Leptostraca and differs from that of the Decapoda.

7. There is no superficial differentiation of the germ layers. Gastrulation takes place by immigration of cells from a blastoporal depression.

8. The cells that wander inwards are mesendodermal in nature. They give rise to yolk cells and the naupliar mesoderm somites.

9. A preantennulary mesoderm somite is formed separately at the anterior end.

10. The post-naupliar ectoderm and mesoderm are teloblastic in origin, and the arrangement of the teloblasts is similar to what is found in *Nebalia.*

11. The mid-gut rudiment is formed by cells which slip in last from the blastopore and it grows by the addition of modified yolk cells.

12. The liver is formed by the folding of the mid-gut epithelium.

13. The formation and growth of the naupliar and trunk mesoderm are described. Segmental cœlomic spaces are formed in the post-naupliar region of the body.

14. No dorsal organ is developed.

15. The latest stage obtained shows four pairs of appendages in the abdomen and a faint pigmentation in the optic rudiments.

16. The paper discusses the results of the investigation in the light of recent work on Crustacean embryology and closes with a consideration of their significance in tracing the affinities of the Stomatopoda.

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14. LIST OF ABBREVIATIONS

ant. .. antenna.
antl. .. antennule.
app. .. appendage.
bl. .. blastomere.
bl.p. .. blastopore.
coel. .. cœlomic space.
c.p. .. caudal papilla.
crp. .. carapace.
d.mes. .. dorsal mesoderm block.
ect. .. ectoderm.
end.pl. .. endoderm plate formed from yolk cells.
e.tel. .. ectodermal teloblasts or their descendants.
g. .. gut.
ht. .. heart.
im.c. .. immigrating cells.
lbr. .. labrum.
liv. .. liver.
liv.ru. .. liver rudiment.
mdb. .. mandible.
mdb.t. .. mandibular tendon.
mdg. .. mid-gut.
mdg.ru. .. mid-gut rudiment.
mes. .. mesoderm.
mes.c. .. mesoderm cells.
mes.tel. .. mesodermal teloblasts or their descendants.
n.c. .. nerve cord.
n.gl. .. nerve gangilla.
nu. .. nucleus.
o. .. oocyte or egg.
opt.ru. .. optic rudiment.
ov.w. .. wall of the ovary.
p.a.m. .. preantennulary mesoderm.
p.aor. .. posterior aorta.
p.b. .. polar body.
pm. .. proctodæum.
rg.ru. .. rectal gland rudiment.
sp. .. nuclear spindle.
stm. .. stomodæum.
stm.d. .. diverticulum from the stomodæum.
v.mes. .. ventral mesoderm block.
y. .. yolk.
y.c. .. yolk cells.

Figs. 1 to 32 have been reduced to ¾ the dimensions given.

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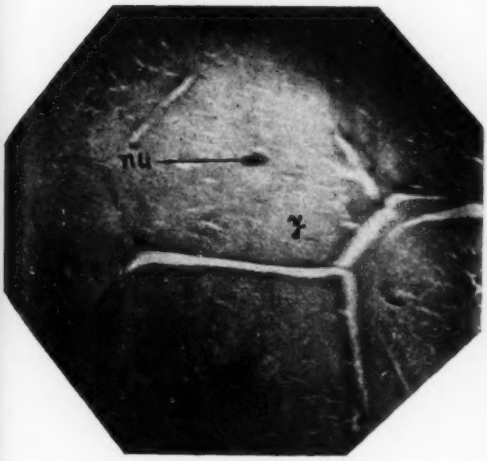
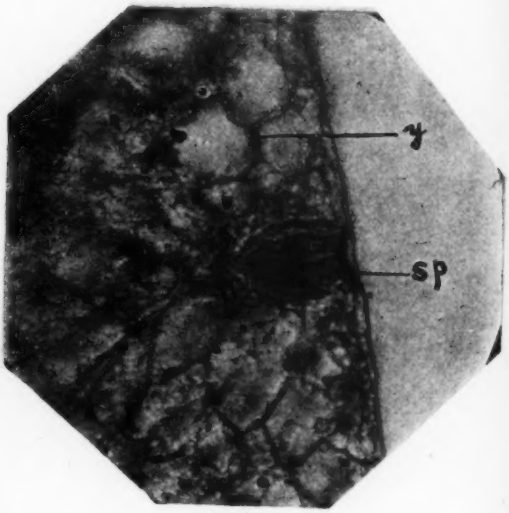


FIG. 33



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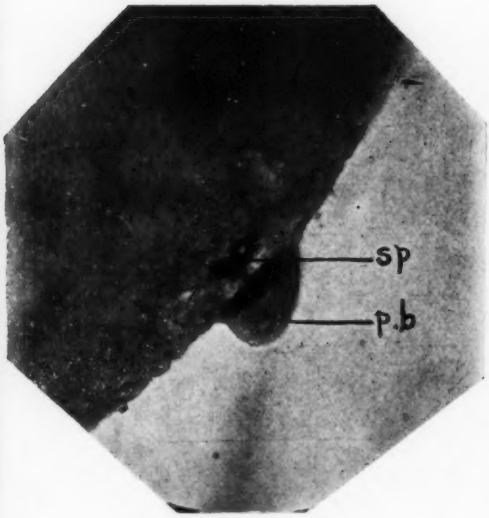

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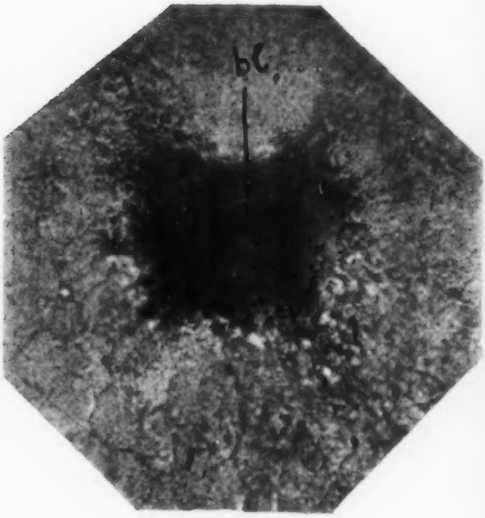
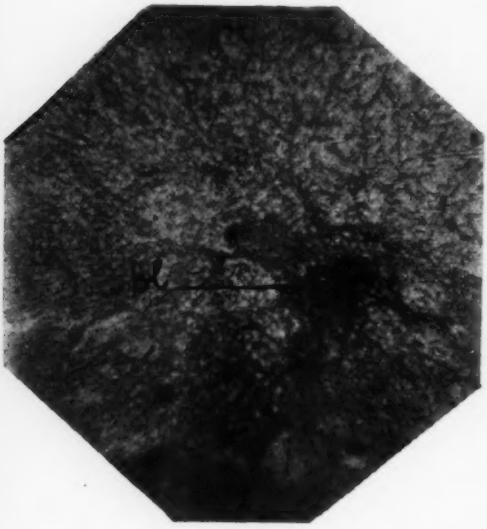


FIG. 36

FIGS. 33-36

FIG. 33. Photomicrograph. *S. wood-masoni*. The fully-grown eggs within the ovary. × 600. FIG. 34. Photomicrograph. *S. raphidea*. The first polar spindle. × 1800. FIG. 35. Photomicrograph. *S. raphidea*. Formation of the first polar body. × 1800. FIG. 36. Photomicrograph. *S. wood-masoni*. A single blastomere about to divide. × 1800.



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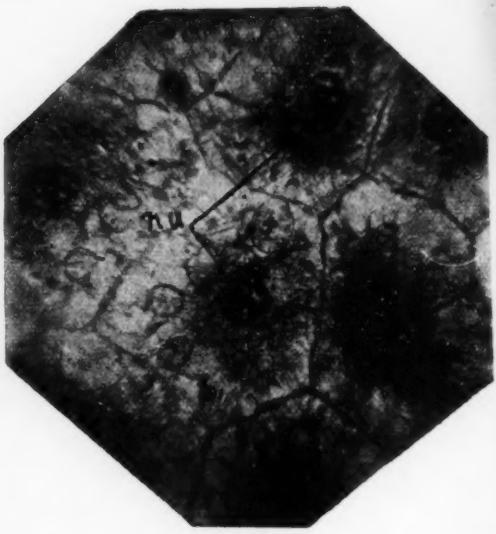

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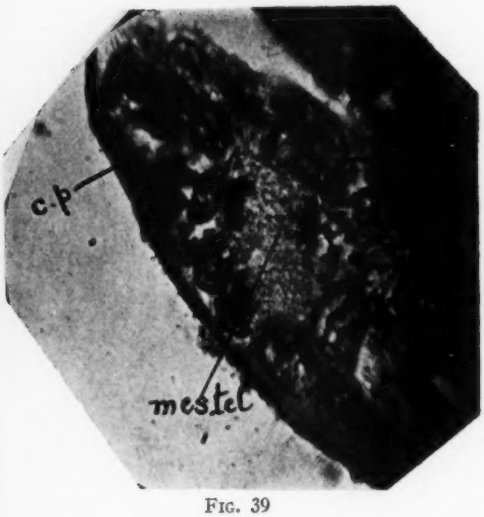

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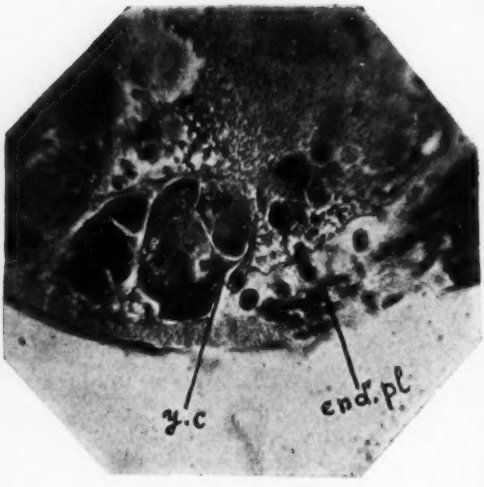

FIG. 40

FIGS. 37–40

FIG. 37. Photomicrograph. *S. raphidea*. A single blastomere of the four-celled stage. × 1800. FIG. 38. Photomicrograph. *S. raphidea*. The blastomeres after they have risen to the surface. × 1800. FIG. 39. Photomicrograph. *S. wood-masoni*. Sagittal section of the tip of the caudal papilla showing the division of the mesodermal teloblasts. × 1200. FIG. 40. Photomicrograph. *S. raphidea*. Section showing the yolk cells getting transformed into the mid-gut epithelium. × 600.

ERRATA

Vol. XIV, No. 3, September 1941, Pp. 310–337.

Plate XI, Fig. 2, *for* " Case No. 2 " *read* " Case No. 3 ".

Page 314, 3rd line from the bottom, *for* " arneth " *read* " Arneth ".

Page 315, *after* " Leucocytic count " *add* " *and* differential count in percentage ".

Page 315, last item in the chart under column " Eosin ", *for* " 3 " *read* " 2 ".

Page 316, 8th line from top, *for* " vanden Bengh reaction " *read* " Vanden Bergh reaction ".

Page 316, Blood Sugar Table, *for* " Cadveric blood " *read* " cadaveric blood ".

Page 317, 5th line from top, *for* " Cadveric Blood " *read* " cadaveric blood ".

Plate XII, Fig. 5, 2nd line, *add* a full stop after " multilobular variety ".

Plate XII, Fig. 5, 2nd line *for* " dilation " *read* " Dilatation ".

Plate XII, Fig. 5, 3rd line, *add* after " the hepatic cells " the words " *are seen* ".

Page 321, 2nd para, 3rd line, *omit* " *are* " in " latter *are* predominate ".

Page 323, 5th para, 8th line, *for* " abscess " *read* " abscesses ".

Page 323, 5th para, in the 10th line, *for* " abscess " *read* " abscesses ".

Page 326, *omit* the 1st para sub-heading " *Conclusion* ".

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